AQUEOUS WASHING OF MECHANICALLY DEBONED CHICKEN MEAT: EFFECT ON CHEMICAL AND FUNCTIONAL CHARACTERISTICS

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BY

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ABSTRACT

Mechanically deboned chicken meat (MDCM) was prepared from backs and necks of freshly slaughtered birds. Samples were then subjected to aqueous washings at various pH conditions using water, 0.5% NaCl or $NaHCO_3$. Approximately 73.9% of the total hemoprotein pigments and 52.1% of total lipids were removed by washing with a sodium bicarbonate solution which resulted also in the best colour improvement in the products. Extraction of these components with water and NaCl solution was less effective. The yield of proteins ranged from 58.1% after one washing with water to 41.7% after washing with water and then with a sodium bicarbonate solution. The amino acid content and protein efficiency ratio (PER) values of washed meats, calculated by an amino acid scoring methodology, were comparable to those of unwashed MDCM.

The concentration of flavour precursors, namely free amino acids and inosine 5′-monophosphate was decreased by over 50% in the washed samples. Similarly, the content of nucleic acids and cholesterol in the corresponding washed samples was reduced by 60.5 and 42.3%, respectively. A slight increase in the content of polyunsaturated fatty acids of phospholipids and content of phospholipids was also noted in the washed MDCM. The cook yield of washed MDCM was enhanced, while a decrease in sulphydryl groups and a proportionate increase in disulphide bonds due to washing was noted. The oxidative stability of washed raw products was less than their unwashed counterpart, however, the cooked washed meats were more resistant to lipid oxidation than the unwashed sample during a 2-week storage at 4 °C.

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LIST OF ABBREVIATIONS

ADP - Adenosine diphosphate

AMP - Adenosine monophosphate

AOAC - Association of Official Analytical Chemists

ATP - Adenosine triphosphate

BHA - Butylated hydroxyanisole

BHT - Butylated hydroxytoluene

DNA - Deoxyribonucleic acid

DTNB - 5,5′-dithiobis(2-nitrobenzoic acid)

FAME - Fatty acid methyl esters

FAO - Food and Agricultural Organization

GC - Gas chromatography

Hb - Hemoglobin

HPLC - High performance liquid chromatography

HUFA - Highly unsaturated fatty acids

HX - Hypoxanthine

ICP - Inductively Coupled Plasma

IMP - Inosine 5′-monophosphate

ISP - Isolated Soy Protein

LPC - Lysophosphatidylcholine

Mb - Myoglobin

MDCM - Mechanically deboned chicken meat

MDPM - Mechanically deboned poultry meat

MDRM - Mechanically deboned red meat

MDTM - Mechanically deboned turkey meat

MW - Molecular Weight

NL - Neutral lipids

PC - Phosphatidylcholine

PE - Phosphatidylethanolamine

PER - Protein efficiency ratio

PL - Phospholipid

PS - Phosphatidylserine

RNA - Ribonucleic acid

SDEW - Spray-dried egg white

SDS - Sodium dodecyl sulphate

SF - Solvent front

SPH - Sphingomyelin

TBA - Thiobarbituric acid

TBHQ - Tertiary-butyl hydroquinone

TLC - Thin-layer chromatography

USDA - United States Department of Agriculture

WHC - Water holding capacity

WHO - World Health Organization

X - Xanthine

This thesis is dedicated to the memory of my grandmother,

Mrs. Agbonaya Oseikhuemen who went to be with the Lord

on December 22, 1992.

CHAPTER 1

INTRODUCTION

Poultry meat constitutes approximately 20% of the meat consumption in the North American diet. Increased popularity of marketable chicken as cut-up parts provides a considerable amount of less preferred parts which include backs and necks, and these are usually processed by mechanical deboning. Mechanical deboning involves separation of bone residues from tissue producing a paste-like product, known as mechanically deboned chicken meat (MDCM). The small particle size of such meat makes it suitable for use in emulsion-type products. Mechanically deboned poultry meat (MDPM) is used as the main ingredient in meat products such as bologna, pimento bologna, frankfurters, patties, cutlets and diced products. However, inclusion of heme and lipid components from bone marrow increases the content of hemoprotein pigments in MDCM, up to three times higher than those in their manually deboned counterparts (Froning and Johnson, 1973; Pikul and Niewiarowicz, 1988). Higher hemoprotein content in MDCM results in a darker colour of the products. However, there is a preference for preparation of products, such as nuggets from white chicken meat. Hemoprotein pigments are also known to catalyze lipid oxidation (Lee *et al.*, 1975) which hastens the development of undesirable flavours during storage of meat. The content of cholesterol and nucleic acids is also elevated in MDCM due to inclusion of bone marrow and spinal cord components. The dark colour and poor keeping quality of MDCM, due to higher contents of hemoproteins and lipids, has hindered the widespread use of MDCM in value-added product

formulations.

Although the nutritional quality of MDCM is comparable to that of manually deboned meats, drawbacks inherent in it necessitates improvement in the quality of MDPM. Young (1975) extracted proteins from MDCM with an aqueous solution at pH 7.0 and an ionic strength of 0.5; the solubilized proteins were recovered by reducing the pH and the ionic strength of the slurry to 4.5 and 0.2, respectively. Centrifugation of deboned meat/water slurry has been shown to reduce the content of pigments and fat and lipid oxidation of MDCM as reflected in 2-thiobarbituric acid (TBA) values (Froning and Johnson, 1973). Hernandez *et al.* (1986) investigated the effect of aqueous washing under different pH conditions on mechanically deboned turkey flakes and found that increasing the pH of the phosphate solution used for washing increased Hunter **L** (lightness) and decreased Hunter **a** (redness) values. Lin and Chen (1989) were able to partially remove lipids and pigments from MDPM by washing with NaCl or phosphate buffers at pH 8.0. Dawson *et al.* (1988) also reported that washing of MDCM with 0.5% bicarbonate solution improved the colour of products better than washing with water or a 0.1% acetate buffer. Aqueous washing of MDCM may provide raw materials which could have potential applications in a wide range of product formulations.

CHAPTER 2

LITERATURE REVIEW

2.1 Chicken production

Meat is a major source of protein in human nutrition. Poultry meat, mainly chicken, contributes to consumers a substantial part of the muscle protein supply. In Canada, the 1991 chicken production was approximately 575 million kg carcass weight (Statistics Canada, 1991). This would in turn provide 7% (40.2 million kg) necks and 8% (46 million kg) backs. However, only 90% of necks and 10% of backs are available for mechanical deboning since the remainder of necks and backs are sold as cut-up parts (Burke, 1992). The 1991 production of chicken in Newfoundland was 7 million kg which represents about 1.2% of the total national production (Statistics Canada, 1991). The production of chicken meat in the United States is approximately 10 times that of Canada.

2.2 Meat deboning

The potential recovery of residual meat from bones and fish frames began in Japan and provided an opportunity for better utilization of recoverable meat. Although the technology has improved tremendously, the mode of action of the early Japanese fish deboner of the 1950s is still the basis of today's deboning technology. Mechanical deboners for poultry were developed in the 1950s in the United States (Field, 1988). Today, deboned products are a well developed segment of the meat industry in Canada and the United States of America.

2.2.1 Types of deboning processes

A further processing step beyond production of cut-up poultry parts is that of boneless products. Generally speaking, deboning as practised in the industry today falls into two categories: hand deboning and mechanical deboning. The chicken breast and thighs are usually hand deboned. This may involve stripping the breast muscles and skin from carcass on eviscerating lines (Hamm *et al.*, 1982). This process is called hot stripping of meat. Hot deboning which involves the removal of breast and thigh muscles and skin after normal evisceration but prior to chilling results in meat which lacks tenderness and this greatly limits potential uses of such meats. A considerable amount of poultry is cooked prior to hand deboning, especially with mature hens where large muscles are kept relatively intact so that they can be chilled and diced for use in soups, salads, or for freeze drying which is generally used for dehydrated soup mixes (Stadelman *et al.*, 1988).

Production of mechanically deboned red meat (MDRM), mechanically deboned poultry meat (MDPM) and minced fish is commonplace. MDRM refers to mechanically deboned beef, pork, lamb and mutton (Field, 1988). For poultry, mechanical deboning is usually employed for recovery of meat residues from parts left after hand deboning. Whole carcass and less preferred bony parts of chicken such as necks and backs may also be used. MDPM is a comminuted product of chicken or turkey which has been processed through an automatic deboner. Utilization of poultry meat has increased with the use of

deboning machines.

2.2.2 Mechanical deboners

Various types of mechanical deboners for processing of poultry parts may be used. The process involves breaking of bones into smaller pieces or grinding followed by forcing the meat through a fine screen or slotted surface. The deboned meat which emerges from the machine is a finely ground, paste-like, product while the bone particles except the very small ones are excluded and expelled as waste. Such bone waste may be used as a component of animal feed, fertilizer or as raw material for production of protein isolates (Froning, 1981; Lawrence *et al.*, 1982). Two types of deboners are usually used.

The sieve-type deboner requires the bones to be preground in a bone grinder. The bone material is forced into the drum separator containing microgrooves that allows the meat to pass through into the inner portion of the drum as bone and connective tissue are retained in the outer part of the drum. The Beehive (Beehive Machinery Inc., Sandt, Utah) deboner belongs to this group. The press-type deboner is hydraulically powered and presses batches of meat with bones attached without grinding against a stationary slotted or grooved surface. The meat and fat are squeezed out through screens or along microgrooves, while bone is retained and then ejected. Examples of the press-type are the Hydrau (Hydrau BV, OSS, Holland) and Protecon (Protecon BV, OSS, Holland) deboners.

2.3 Product yield

In general, yields of meat upon deboning are influenced by the amount of muscle attached to the bones to be mechanically separated, the type of deboner and setting. Like hand deboning, bones with higher amounts of meat attached give higher yields. Yields of MDPM from various parts of the carcass usually range from 55 to 70% depending on the part being deboned (Field, 1988). The proportion of bone to meat in the original product to be deboned, the design of the deboning equipment and size of the orifices through which the meat is forced are factors also influencing the product yield. Vadehra *et al.* (1972) reported that most of the equipment available then for deboning gave yields from 40 to 60% meat and the residue contains 63% moisture, 14 to 16% protein, 12 to 14% lipid and 4 to 5% ash. Ahn *et al.* (1981) reported that deboning of domestic broilers and spent layers after primary hand deboning gives a yield of 45% of MDCM (Table 2.1). The yield of deboned meat may also be affected by the deboner settings. Kumar and Wismer-Pedersen (1983) obtained a yield of 87% deboned product at a particular setting and 10% less yield using a reduced pressure setting.

2.4 Structural characteristics and composition of MDPM

The shearing action of the mechanical deboning process causes considerable cellular and tissue disruption. The deboned meat is therefore a finely ground, paste-like, product in which the myofibrils are highly fragmented (Froning, 1981). Some of the

Table 2.1 Yield of meat from deboned chicken carcasses (%)[1].

Sample	HDCM[2]	MDCM[3]	Total
Broiler	33.0	49.5	82.5
Spent layer	36.6	40.8	77.4
Average	34.7	45.2	79.9

Adapted from Ahn *et al.*, (1981). [1]Meat yield (%) = (Deboned meat/carcass weight)x100.

[2]HDCM, Hand deboned chicken meat.

[3]MDCM, Mechanically deboned chicken meat. The parts left after hand deboning were mechanically deboned.

problems experienced with MDPM are related to the loss of integrity of myofibrils during the deboning process.

In a study on the ultrastructure of MDPM using a deboner with screen sizes of 0.1575, 0.1016 and 0.0508 cm, Schnell *et al.* (1974) reported that the characteristic size of the myofibrils was altered by the smaller screen sizes. Breaks were observed at the Z or M bands, indicating that the shearing process reduces the length of the myofibrils. Vadehra and Baker (1970) also examined the structure of deboned meat histologically and observed no intact muscle fibre in commercially deboned necks and backs. New models of mechanical deboners have been developed which produce deboned meat with adequate texture when used as a primary ingredient in meat balls, nuggets and patties (Stadelman *et al.*, 1988).

The proximate composition of MDPM varies considerably with the type of raw material used, the design of the deboner and procedures adopted for processing (Keshri *et al.*, 1981). However, Orr and Wogar (1979) reported that the source of raw material has a greater influence on the composition of MDPM than the deboning machine used. Therefore, one reason for the changing composition of MDPM may be due to the raw material that is used for production of MDPM. At the onset of poultry deboning, only those parts left after portioning of the cut-up parts were mechanically deboned (Hamm and Young, 1983). These parts included the narrow back bone strips and necks, with or without skin, and possibly wing tips. The whole bony skeleton or any combination of

bony parts may be used as raw material (Hamm and Young, 1983).

Various authors have reported variability in the composition of commercial MDPM. Many investigators have reported a lower protein and a higher fat content in mechanically deboned meat as compared with manually deboned meat (Froning *et al.*, 1971; Grunden *et al.*, 1972; Froning and Johnson, 1973). Essary and Ritchey (1968) noted that mechanically deboned turkey light meat contained 15.0% fat, 13.3% protein and 67.5% moisture, whereas, dark meat resulted in a product with 12.4% fat, 11.8% protein and 70.2% moisture. The moisture content of MDPM is usually higher than in manually deboned products since the deboning process involves compression of the raw material, which favours the extraction of moisture from bone marrow along with the deboned meat (Grunden *et al.*, 1972). MacNeil *et al.* (1978) reported the compositional data of MDCM obtained from different parts of chicken. The moisture, protein, lipid and ash contents were found to vary with the parts used for deboning. The composition of deboned products may also be influenced by the skin content of the raw material. Satterlee *et al.* (1971) observed that as the skin content on chicken broiler backs increased in relation to muscle and bone content, the fat content of deboned meat increased while the moisture and protein content decreased (Table 2.2). Various authors have reported that the ash content, which is an indication of the amount of minerals, varies with the source of raw material, type of deboner and age of bird. Hamm and Young (1983) reported a 0.5% increase in the ash content of MDPM as compared with that of the hand deboned samples.

Table 2.2 Effect of skin content prior to deboning on the chemical composition of MDCM[1].

Skin content %	Moisture %	Fat %	Protein %	Collagen mg/g meat
0	66.1	15.3	14.5	11.8
16.8	59.8	24.6	12.0	12.3
28.9	55.8	29.8	11.4	10.9
38.9	53.7	33.6	10.2	11.8

[1]Adapted from Satterlee *et al.*, (1971) and Froning *et al.*, (1973). The total content of moisture, fat and protein in samples was 95.9-97.5%. Content of minerals was not reported.

2.5 Minerals, vitamins and bone content of MDPM

The mineral content and composition of MDPM have been of concern to consumers. Considerable research has been done to determine the type and quantities of minerals present in MDPM. Mast *et al.* (1982) found a variation in calcium content (ranging from 0.12 to 0.35%) of mechanically deboned broiler backs and necks depending on the type of deboning machine used, but found no significant differences in proximate composition of products. Murphy *et al.* (1979) and Grunden and MacNeil (1973) found a higher level of calcium in mechanically deboned spent fowl than that present in younger birds. This reflects the higher degree of fragmentation of bones of older birds during deboning thereby resulting in an increased level of bone particles. Several other minerals, especially the heavy metals, have been studied because of their health-related and safety implications. Murphy *et al.* (1979) have concluded that these minerals do not pose any health hazard to humans when present in MDPM. Essary (1979) also analyzed several minerals from various types of MDPM and the levels detected were found to be safe for human consumption. He also observed a wide variation in mineral content of mechanically deboned broiler parts and noted that all elements analyzed were higher in mechanically deboned turkey meat (MDTM) than hand deboned broiler meat. Calcium and magnesium were present at four and two times higher amounts, respectively, in MDTM. The higher calcium content in MDTM could be attributed to a higher percentage of bone in turkey as compared to chicken meat. However, the mineral content in these

meats were at levels that are not considered unsafe for human consumption. Higher levels of calcium in MDCM (53-91 mg/100 g) as compared with those in hand-deboned samples (17-34 mg/100 g) has been reported (Ang and Hamm, 1982). These authors also reported a higher iron and a lower copper level in mechanically deboned as compared with hand deboned necks. The apparent lower copper content in MDCM may be due to the dilution effect caused by incorporation of additional fat into the product and the increased iron content is perhaps due to the increase in the hemoprotein content of MDCM. Increased iron content in MDCM might make such meat more prone to rancidity development during storage as metal ions are known catalysts of lipid oxidation.

The content of vitamins in MDPM was first reported by Ang and Hamm (1982). They found that MDCM (with skin) contained far less vitamin B_6, niacin and pantothenic acid and slightly less thiamine than did manually deboned broiler breast and thigh meat. The necks and back meats, however, contained more riboflavin than breast meat while there were no significant differences in the levels of these vitamins in mechanically deboned samples as compared with corresponding manually deboned meat. The storage effects on thiamine and riboflavin content of MDCM and its further processed products, frankfurters and bolognas, were evaluated by Ang (1986). There was little loss of these vitamins during storage of products at 2 or -18 °C for up to 180 days.

The possible presence of bone fragments in MDPM is also of concern (Froning, 1981). Studies, however, indicate that bone fragments in MDPM are smaller than in hand

deboned meat (Froning, 1979) and their presence in meat products should not pose any undue problems. Sizes of bone fragments in MDCM from uncooked fowl have been reported to be smaller than when the birds were cooked prior to mechanical deboning (Froning *et al.*, 1981).

2.6 Hemoprotein content and colour characteristics

The bone marrow is another component affecting the final composition of MDPM. This component of the chicken carcass contains substantial quantities of hemoglobin which is released during mechanical deboning (Froning, 1981). Consequently, hemoproteins are present in elevated amounts in mechanically deboned meats as compared with their manually deboned counterparts. Froning and Johnson (1973) observed that mechanical deboning of whole carcasses tripled the hemoglobin content of the resultant deboned fowl meat. Pikul *et al.* (1988), however, reported varying amounts of heme pigments in samples obtained from different chicken parts as well as deboning procedures. The total hemoprotein content in the samples ranged from 2.8 to 4.2 mg/g. Larger amounts of pigment were present in MDCM from carcass frames after removal of breast and leg muscles. This was attributed to the presence of a higher percentage of bones, and hence hemoproteins, in the starting material.

The high levels of hemoprotein present in MDCM results in products which have a dark colour. Shortly after deboning, hemoglobin from the marrow and myoglobin from

the muscles are in the form of oxyhemoglobin and oxymyoglobin as a result of oxygenation. The high amounts of these compounds in freshly produced MDPM can become a problem during storage because they are oxidized to produce brown, green and grey colours (Janky and Froning, 1975). The resultant dark meat is less desirable as an ingredient for restructured meat products which may require a light-coloured meat source (Froning, 1976). Hemoproteins are also catalysts of lipid oxidation in MDCM (Lee *et al.*, 1975), hence MDCM is more susceptible to lipid oxidation than manually deboned chicken meat. Incorporation of high amounts of skin in MDCM has been reported to result in a significant decrease in the total heme pigment and a lighter meat is produced due to dilution of pigments in the deboned meat (Froning *et al.*, 1973).

2.7 Amino acid composition and protein quality

The protein quality of MDPM must be maintained if it is to replace hand deboned meats in various products. Several studies have been carried out to investigate the protein quality of MDPM. The amino acid levels in MDPM were similar to those of the starting material or to manually deboned meat (Essary and Ritchey, 1968; Hamm and Young, 1983; Sarwar *et al.*, 1985). However, Sarwar *et al.* (1985) reported that manually deboned breast and leg meats contained more essential amino acids, less hydroxyproline and had higher protein quality in contrast to MDPM from necks, backs and frames which generally had similar compositions as those in the starting materials. Satterlee *et al.*

(1971) and Field and Riley (1974) reported that collagenous proteins from skin and tendon are largely removed during mechanical deboning of raw meat, leaving only 2-4% in the mechanically processed meat. Different deboner settings influenced the level of collagen in the deboned products. This was also reported by Kumar and Wismer-Pedersen (1983) who observed a higher collagen content in MDCM produced from a reduced pressure setting as compared with product obtained using a higher pressure deboner setting, indicating extrusion of collagenous tissues in MDCM at reduced pressure settings.

Biological evaluation of protein quality of MDPM as expressed by protein efficiency ratio (PER) has been reported by various authors. Although there were slight variations in the PER values obtained in different studies, the protein quality of MDPM was generally comparable to that of hand deboned meat and a standard casein sample. MacNeil *et al.* (1978) reported that the PER values of raw mechanically deboned broiler meat were comparable to a standard casein diet. They reported PER values of 2.65 and 2.47 for skinless necks and a combination of skinless necks and backs, respectively. Results indicated that deboned meat from skinless necks was superior to meat from skinless backs. The difference in PER values was thought to originate from differences in the content of total lipids and alteration of proteins due to rancidity. The effect of antioxidants on PER values of MDPM has also been investigated. Turkey samples treated with an antioxidant had a significantly higher PER value than the 2.5 for standard casein.

Samples devoid of antioxidants had a lower value, possibly due to protein alteration caused by rancidity.

Lee *et al.* (1978) reported a marginal difference in PER values of hand deboned chicken meat and MDCM, but in contrast, cooked MDCM showed a lower PER value than that of a cooked hand deboned chicken. This is a result of a higher collagen content in cooked MDCM than its cooked manually deboned counterpart. The lower PER may be attributed to gelatinization of the chicken skin during cooking and its subsequent greater extrusion with meat during mechanical deboning. Similar results were obtained in mechanically deboned fowl meat where higher content of nonessential amino acids in cooked samples were noted (Babji *et al.*, 1980). However, the amino acid levels of MDCM reported by these authors were comparable to the FAO/WHO provisional scoring amino acid patterns (Table 2.3). The total content of amino acids reported by these authors was 103 g/100 g protein. Possible explanations for this overestimation have been provided by Regenstein and Regenstein (1984). Sulphur-containing amino acids (cystine and methionine) and lysine were also reported to be lower in the cooked MDCM as compared to its raw counterpart. These amino acids are heat labile and are easily degraded at elevated temperatures. Studies on protein quality of MDPM indicated that mechanical deboning of poultry meat does not lower the protein quality in the deboned products. Hence, MDPM can be used in processed meat formulations without compromising protein quality. Use of appropriate deboners which meet government

Table 2.3 Amino acid content of mechanically deboned chicken meat (MDCM) and the FAO/WHO provisional amino acid scoring patterns, g/100 g protein.

Amino acids	Raw MDCM[1]	FAO/WHO[2]
Essential:		
Isoleucine	3.91	4.00
Leucine	7.99	7.04
Lysine	11.29	5.44
Methionine + Cystine	3.98	3.52
Phenylalanine + Tyrosine	7.28	6.08
Threonine	4.78	4.00
Tryptophan	1.12	0.96
Valine	4.05	4.96
Histidine	3.12	...
Nonessential:		
Alanine	7.37	...
Glycine	7.43	...
Proline	4.93	...
Serine	4.51	...
Aspartic acid[3]	9.75	...
Glutamic acid[4]	15.32	...
Arginine	6.34	...

[1] MDCM (Mechanically deboned chicken meat from backs and necks). Adapted from Babji *et al.*, (1980).

[2] Food and Agricultural Organization/World Health Organization, (1973). A factor of 6.25 g of protein/g of nitrogen was used for converting nitrogen to protein.

[3]Determined as aspartic acid + asparagine.

[4]Determined as glutamic acid + glutamine.

regulations and standards will lower the collagen and skin contents of MDPM and hence improve the protein quality of the deboned products.

2.8 Cholesterol content

Cholesterol is a minor food component which many consumers of animal products are often concerned about because of its association with cardiovascular disease (Gur, 1984). Inclusion of the spinal cord in MDPM results in elevated cholesterol content of such products in contrast to their manually deboned counterparts. Moerck and Ball (1974) reported that the cholesterol content of raw MDPM from chicken backs and necks was 560 mg/100 g, while Jantawat and Dawson (1979) reported 74 mg cholesterol/100 g fowl meat. Ang and Hamm (1982), however, investigated the effect of deboning on the cholesterol content of broiler parts and reported it to be 95 mg/100 g in mechanically deboned samples from whole backs. This was about 14% higher than that in the hand deboned meat from the same parts. This increase was attributed to the inclusion of both the back fat and the spinal cord in the mechanically deboned samples. Schuler (1985) found that the cholesterol levels in MDPM from necks range from 134 to 148 mg/100 g with the higher value being from necks with skin attached. MDCM obtained from chicken parts with no spinal cord had a lower amount of cholesterol. Deboned meat from chicken backs and breasts contained 98 and 97 mg cholesterol/100 g samples, respectively. The cholesterol content of manually deboned chicken breast has

been reported to be 67 mg/100 g (Feeley *et al.*, 1972). A United States Department of Agriculture (USDA) panel charged with the analysis of the health and safety aspects of mechanically deboned meat, however, reported the cholesterol content of MDCM ranged from 28 to 202 mg/100 g tissue (Kolbye *et al.*, 1977). Various workers have reported that the cholesterol content of mechanically deboned muscle foods to be 68 mg/100 g for cod (Krzynowek *et al.*, 1984), 99 mg/100 g for seal (Shahidi and Synowiecki, 1991) and 153 mg/100 g for beef (Kunsman *et al.*, 1981).

2.9 Nucleic acids

Bone marrow is the main site of blood cell formation and this, therefore, accounts for the high content of nucleic acids, which are involved in protein synthesis. This results in elevated amounts of nucleic acids in mechanically deboned meats as compared with their manually deboned counterparts. The purine moieties of nucleic acids are degraded to uric acid which has low solubility under physiological conditions (Waslein *et al.*, 1968). The high level of nucleic acids may therefore pose a health risk to gouty individuals who produce high levels of uric acid due to an inborn error of metabolism.

Limited data is available on the nucleic acid/purine content of MDPM. Murphy *et al.* (1979) concluded that deboned products did not present any health hazard at any level in terms of their purine content. They found that total purine content of MDPM did

not differ from that of manually deboned meat. Their data also showed that hypoxanthine was actually lower in MDPM than in hand deboned products, and adenine in MDPM did not differ from that of hand deboned meat. However, Young (1980) reported higher levels of purines expressed per unit nitrogen in mechanically deboned broiler meat as compared with conventional broiler tissues. Total nucleic acid levels have also been reported to be higher in mechanically deboned beef, veal (Savaiano *et al.*, 1983) and seal meat (Synowiecki and Shahidi, 1992) as compared with their manually deboned counterparts. Arusa *et al.* (1981) reported elevated nucleic acids in bone marrow which increased the amounts of these compounds in mechanically deboned beef as compared with their manually deboned counterpart. Young (1985) assessed the nucleic acid and purine content of the bone residues of poultry from a mechanical deboning machine. He found that the levels of these compounds per gram protein in bone residues was the same or lower than those in edible meat. Therefore, recovered protein from bone residues of poultry may be used, without any concern, in food formulations.

2.10 Functional properties

Functional properties are those physical and chemical properties which affect the behaviour of proteins and other components in meat system during processing, storage, preparation and consumption (Kinsella, 1982a). These characteristics influence the quality and sensory attributes of foods and hence are most important in determining the

usefulness of proteins in food systems. Proteins contribute significantly to the functional behaviour and quality of foods and with the increased interest in processed and formed meat products, greater importance is being placed on the functional properties of the protein components. The most important functional properties of meat protein are solubility, emulsification capacity, gelation and water-binding (Kinsella, 1982b).

Emulsified meat products prepared from MDPM are influenced by the formation of a continuous matrix throughout the product which is mainly due to its protein components. Formation of a matrix determines the texture, juiciness and general mouthfeel of products (Kinsella, 1982a). Emulsifying, binding and gelling properties of muscle proteins following processing and cooking are generally considered important (Schmidt *et al.*, 1981).

Water holding capacity (WHC) also plays an important role in the processing, storage, cooking and freezing of meat because it relates to weight loss and quality of the finished product (Field, 1988). The WHC of MDPM is enhanced because these products have a higher pH than the hand deboned meat. Increases in pH are due to the incorporation of red marrow, which has a pH in the range of 6.8 to 7.4 (Field, 1981). As the pH of meat is increased, WHC increases as minimum WHC occurs at pH 5.2-5.5 because protein molecules contained within the filament systems attract each other and squeeze out water molecules; whereas, above this pH range, there is a repulsion between different protein molecules and this allows entrapment of water in the protein network

which results in their swelling (Asghar and Pearson, 1980). The term "water retention properties" may be used in place of WHC. The water retention properties fall into two categories: (1) Expressible moisture (EM) which is a measure of actual loss of moisture from a sample due to application of force, for example, pressing in a hydraulic press or centrifugation. (2) Water-binding potential (WBP) which measures the maximum amount of water that a sample can hold when excess aqueous solution was added, at a paticular set of solution and centrifugation conditions (Regenstein and Regenstein, 1984).

The relationship between the source and type of mechanically deboned poultry parts and functional properties has been studied. The proportion of skin remaining on the necks and backs following cutting and trimming has been implicated to influence the functional properties of MDPM. Froning *et al.* (1973) reported that presence of larger amounts of skin resulted in a significant decrease in emulsion stability of MDPM. However, Ahn *et al.* (1981) reported that emulsifying capacity of MDCM was only 70% that of its hand deboned counterpart, but a higher emulsion stability was noticed (expressed per g of protein). This was attributed to a higher proportion of salt-soluble proteins in MDCM. Mast *et al.* (1982) studied the effect of different deboning machines (Paoli, Beehive, Yieldmaster and Protecon) on functional characteristics of MDCM and found that meat from Protecon and Beehive machines had a higher emulsifying capacity and produced the most stable emulsions. Water holding capacity of the deboned meat was also affected by type of deboning machine used as meat obtained from the Paoli

deboner retained the highest percentage of the original moisture.

Orr and Wogar (1979) reported that MDCM produced from necks and backs and from various industrial sources exhibited significantly different emulsifying capacity, water holding capacity, emulsion stability, fat and moisture content. Functional attributes of meat are also influenced by the relative proportion of connective tissue and myofibrillar proteins in the meat system. McMahon and Dawson (1976) determined the amount of salt-soluble proteins in hand deboned and mechanically deboned turkey meat. The percentage of salt-soluble proteins was somewhat lower in mechanically deboned meat than in hand deboned products, while emulsification capacity was superior in the hand deboned meat. However, water holding capacity measured as % moisture loss during heating and water binding capacity expressed as the % swell due to absorbed water, were higher in mechanically deboned meat as compared to its hand deboned counterpart.

Schnell *et al.* (1973) observed that increasing the amount of skin added to a frankfurter formula increased the fat content, tenderness and viscosity, and decreased emulsion stability. In a related study, Mayfield *et al.* (1978) utilized a temperature-controlled, capillary extrusion viscometer to compare meat batters prepared from MDPM. Batters with higher protein content (12%) were more viscous and had better emulsion stability than those containing less protein. Viscosity of the meat batter also increased with higher levels of fats, but less stable batters were encountered at high fat levels.

Brown and Toledo (1975) studied the relationship between chopping temperature and fat and water binding in comminuted meat batters. They reported an inverse relationship between fat and water binding. The maximum binding occurred at a temperature range of 15-22 °C. Increases in the amount of lipid originating from marrow in MDCM can also influence emulsion properties because bone marrow lipid has more polyunsaturated fatty acids with a lower melting point than lipids in hand deboned meat (Moerck and Ball, 1973). However, Maurer (1973) reported that mechanically deboned broiler backs and necks have similar emulsifying capacities when compared with their hand deboned counterparts. Furthermore, a combination of mechanically deboned spent hen backs, necks and wings and hand deboned breasts, legs and thighs gave high emulsifying and water holding capacities, and hence it may be desirable to use such a combination in emulsion products (Maurer, 1973). Cooking losses in processed meat products are usually influenced by water holding capacity and emulsifying capacity parameters. Mast and McNeil (1976) demonstrated that certain functional properties of MDPM, such as emulsifying capacity and water holding capacity, were impaired at higher temperatures such as those encountered in heat pasteurization.

2.11 Lipid oxidation

2.11.1 General overview

Lipid oxidation is a major cause of quality deterioration of meat especially during storage. Oxidative reactions are responsible for changes in colour, flavour, texture and

nutritional value due to destruction of vitamins A, D and E and essential fatty acids (e.g., linoleic acid) in both fresh and cooked muscles (Dziezak, 1986). These reaction are generally catalyzed by many factors which include oxygen, heat, light, heavy metals, pigments and alkaline conditions. Oxidation of bright red oxymyoglobin leads to the formation of undesirable brown metmyoglobin pigments. Muscle lipids can also undergo oxidation during storage leading to the formation of rancid odour and flavour in products.

Autocatalytic oxidation of lipids involves a free radical chain mechanism which involves the three stages of initiation, propagation and termination. While hydroperoxides, the primary products of lipid oxidation, are odourless and tasteless, their breakdown products such as aldehydes, ketones, alcohols, esters, furans and lactones (Frankel, 1984) are responsible for the occurrence of off-flavours in oxidized lipid-containing foods. Some of the secondary oxidation products may also be toxic (Pearson, 1981).

2.11.2 Factors affecting the oxidative stability of MDPM

2.11.2.1 Lipid composition and hemoproteins

Mechanically deboned meats are more susceptible to lipid oxidation because of the high proportion of unsaturated lipids present in the bone marrow and an increased hemoprotein content as compared with hand deboned meat. In addition, increased aeration during the deboning process and greater cell disruption and increased contact of meat lipids with prooxidants (such as metals and hemoproteins) may also hasten oxidative deterioration of MDPM (Moerck and Ball, 1974; Froning, 1981). Schnell *et al.* (1971)

studied the effect of particle size of MDCM on the oxidative state of products. Oxidation of MDCM increased substantially for samples with the smallest particle size and this was attributed to greater cell disruption and release of heme pigments as well as larger surface area and hence increased exposure to oxygen during the preparation steps.

Igene *et al.* (1980) showed that both neutral triacylglycerols and phospholipids are involved in quality deterioration of meats; however, phospholipids were most affected. Meanwhile, sensitivity of neutral lipids to oxidation was dependent on their degree of unsaturation and the length of frozen storage. Moerck and Ball (1974) also reported that the phospholipid fraction was the major substrate of autoxidative deterioration in MDPM due to incorporation of high levels of unsaturated fatty acids from bone marrow lipids. Siegel and Latimer (1971) reported that chicken bone marrow contains high levels of unsaturated fatty acids. Moerck and Ball (1973) also reported higher chain length phospholipids in bone marrow as compared to other tissues, with linoleic and arachidonic acids as the predominant polyunsaturated fatty acids.

The catalytic effect of heme compounds on lipid oxidation of meat systems is now a generally accepted phenomenon (Igene *et al.*, 1979), however, some heme derivatives may possess antioxidant properties (Kanner *et al.*, 1980; Shahidi *et al.*, 1987). An attempt to characterize the mechanism of lipid oxidation in MDCM was made by Lee *et al.* (1975). They found that the catalytic effect of MDCM homogenate was greater at neutral and alkaline pH and concluded that hemoproteins were the predominant catalysts of lipid

oxidation. However, they also suggested that the extent of lipid oxidation occurring in muscles may be influenced by the ratio of hemoprotein to unsaturated fatty acids. At a low linoleate to hematin ratio, inhibition of lipid oxidation occurred.

The high pressure conditions necessary for mechanical deboning may increase contact with metals of the equipment and higher temperatures caused by frictional forces may result in increased oxidation of both heme and lipid components (Froning, 1981). Mast *et al.* (1982) reported that MDPM obtained from different deboners showed different patterns of oxidative stability during storage. Barbut *et al.* (1989) further reported that higher head pressure types of mechanical deboner resulted in lower fat and higher iron content than lower pressure types and products obtained from the former had the slowest rate of oxidation. Pikul and Niewiarowicz (1988) also reported that oxidative changes of MDCM were most intensive in samples which contained more heme pigments and iron than samples which contained lower amounts of these components during 23 wk of frozen storage at -18 °C as indicated by significant differences in peroxide numbers after 5 wk and malonaldehyde concentrations after 9 wk, of these two groups of samples .

2.12 Control of lipid oxidation

2.12.1 Use of antioxidants

Due to the rapid deterioration of MDCM due to lipid oxidation, several investigators have attempted to maintain desirable flavour attributes using various

antioxidants. Antioxidants can help retard lipid oxidation in both hand deboned and mechanically separated meats. Without antioxidants, 2-thiobarbituric acid (TBA) values for both products rose dramatically between 1 and 5 wk of storage at 4 °C (Field, 1988). Froning (1973) observed a retardation in lipid oxidation of deboned fowl meat obtained from carcasses which were chilled in 6% commercial polyphosphate (Kena®) in ice slush overnight prior to mechanical deboning. The deboned meat had lower TBA values than the control after 2 mo storage at -29 °C. The commercial polyphosphate protected the product against oxidative changes during the deboning cycle and subsequent frozen storage. In a study of a number of potential antioxidants, MacNeil *et al.* (1973) noted that rosemary extract, sodium polyphosphate, and butylated hydroxyanisole (BHA) together with citric acid were effective antioxidants in simulated MDPM (85% meat and 15% skin and flavouring compounds). Sensory and TBA results were used to assess antioxidant capabilities of the additive systems used. They concluded that compounds tested were effective in retarding lipid oxidation and the use of a 0.01% rosemary extract improved flavour of simulated MDPM.

Moerck and Ball (1974) reported that addition of Tenox II (20% BHA + 6% propyl gallate + 4% citric acid in propylene glycol) at 0.01% of fat content of MDPM extended the induction period of lipid oxidation at 4 °C. Samples treated with Tenox II had lower TBA values than the untreated samples. Several studies have shown that BHA and t-butyl hydroquinone (TBHQ) reduced oxidative rancidity in ground beef, pork and

restructured beef/pork steaks (Greene *et al.*, 1971; Chastain *et al.*, 1982; Shahidi *et al.*, 1987; Wheeler *et al.*, 1990). The TBA value of the treated samples was always less than 1.0 mg malonaldehyde equivalents/kg meat over the test period (5 wk at 4 °C or 153 days at -20 °C).

Although synthetic antioxidants have been used successfully to retard lipid oxidation and warmed-over flavour (WOF) development in meat systems, consumers' concern about the use of synthetic chemicals in foods has led to studies on potential application of antioxidants from natural sources. Resurreccion and Reynolds (1990) recently studied the effect of natural tocopherols and extracts of rosemary on oxidative stability of chicken and pork frankfurters and reported that these antioxidants were as effective as BHA/butylated hydroxytoluene (BHT) in retarding lipid oxidation in the products. Sodium tripolyphosphate/oleoresin rosemary has also been reported to effectively retard oxidative degradation of polyunsaturated fatty acids in chicken nuggets during frozen storage (Lai *et al.*, 1991).

2.12.2 Effect of CO_2 and N_2 cooling and vacuum packaging

A considerable amount of heat is generated with the use of certain mechanical deboners and this may initiate lipid oxidation and the development of rancid flavour. Use of CO_2 or N_2 by processors to chill and freeze MDPM has been practiced (Cunningham and Mugler, 1974). However, an improved model of the Protecon deboner which utilizes

a plate and press of reduced width has been produced. Use of this equipment results in less meat coming in contact with the machine and hence reduces heat build-up in the deboned meat (Stadelman *et al.*, 1988).

Uebersax *et al.* (1977) precooled MDCM with "CO_2-snow" using a tumbling process. This process, however, enhanced lipid oxidation while vacuum packing of MDCM significantly retarded it. It has also been observed that deboned meat mixed in air and CO_2 had higher TBA values than those found in the control meat which was mixed under nitrogen (Uebersax *et al.*, 1978). Mast *et al.* (1979) also observed that CO_2 may contribute to the development of oxidative rancidity in MDPM as evidenced by elevated TBA and peroxide values. Jurdi *et al.* (1980) also reported that high-fat MDCM exposed to 100% CO_2, 30% CO_2 or air and held at -20 °C for 2 mo had higher TBA values over the storage period, but meat held under N_2 had consistently lower TBA values. These authors also observed that a N_2 atmosphere was most effective in retarding oxidative rancidity when samples were stored at 5 °C for 10 days. Liquid N_2 and CO_2 "snow" were used by MacNeil and Mast (1980) to chill mechanically deboned spent layer meat which was then held either at 2 °C for 8 days or at -18 °C for up to 6 mo. CO_2-treated meat was found to have a higher TBA value than liquid N_2-treated sample stored at 2 °C for 8 days, however, no differences in TBA values for samples stored at -18 °C were evident. On the other hand, Barbut *et al.* (1990) reported that rapid freezing of MDCM with CO_2 immediately after deboning did not adversely affect the rate of lipid

oxidation unlike the use of CO_2 for prechilling. McNeill *et al.* (1987) studied the effect of modified atmosphere on oxidative stability of frozen MDPM. They reported that both vacuum packaged and N_2-blended meats were more resistant to lipid oxidation than the control without modified atmosphere. Jantawat and Dawson (1980) investigated the effect of N_2 or CO_2 gases and vacuum packaging on MDCM and mechanically deboned turkey meat (MDTM) frozen at -18 °C. They also reported that vacuum and N_2 packaged samples gave significantly higher unsaturation ratios and lower TBA numbers than CO_2 packaged samples. Use of modified atmospheres such as N_2, although they retard lipid oxidation during storage, do not work as well as freezing of samples immediately after such gas treatment.

2.13 Microbiological properties

The mechanical deboning process causes considerable maceration of meat resulting in smaller particle sizes with increased surface area and release of cellular fluids rich in nutrients, which provide a suitable medium for bacterial growth. In addition, improperly cleaned and maintained equipment, temperature increase due to friction generated during deboning and higher pH values of MDPM than those of hand deboned meats may promote microbial growth (Swingler, 1984; Chant *et al.*, 1977). Therefore, proper storage conditions such as immediate freezing of MDPM to retard proliferation of microorganisms in MDPM is necessary.

Ostovar *et al.* (1971) have pointed out that the microbiological quality of deboned meat may deteriorate if the carcass parts are not kept cold and deboned soon after removal from the carcass. These workers estimated total counts, faecal coliforms, *Salmonellae*, *Clostridium perfringens*, coagulase-positive *Staphylococci* and psychrotolerant microorganisms in MDCM from broiler necks and backs, whole fowl and turkey racks. Freezing resulted in a substantial reduction of faecal coliforms. In a related study, Maxcy *et al.* (1973) found that similar microorganisms were present in frozen and fresh MDPM and hand deboned products with *Bacillus* species as the dominant bacteria. The microbial quality of such products remained essentially the same during storage at -20 °C for 7 wk. The levels of aerobic organisms and the incidence of *Clostridium perfringens*, vegetative cells and spores in mechanically deboned chicken backs and necks stored at -23 °C was investigated by Lillard (1977) who reported that frozen storage significantly reduced the incidence and levels of vegetative cells and spores but did not affect the level of aerobic organisms. It was concluded that good handling procedures and adequate cooking temperatures prevent any undue hazard in using MDPM in food products. Moreover, when raw materials originate from more than one plant, special attention should be paid to handling procedures employed.

Studies have been done on possible ways of prolonging the microbial shelf-life of MDPM. Pasteurization and use of antioxidants (rosemary spice extract, BHA and citric acid) were reported to have lowered bacterial counts in MDPM and hence extended their

shelf-life (Young and Lyon, 1973; Mast and MacNeil, 1975; MacNeil *et al.*, 1973). CO_2 and N_2 have been observed to have repressive effects on total aerobic and anaerobic bacterial numbers, respectively (Jurdi *et al.*, 1980). Lactic acid bacteria (LAB) which repress bacterial activity by means of acid production, hydrogen peroxide formation, antibiotics and bacteriocin secretion, have also been reported to control spoilage and pathogenic organisms in cooked MDPM (Raccach and Baker, 1978a, 1978b). Studies on microbial quality of MDPM have concluded that good manufacturing practices and strict quality control program including good sanitary/ hygienic conditions are important criteria which eliminate any microbial problem in products.

2.14 Methods of improving the quality of MDPM

Although MDPM is used in emulsified and other processed meats, attempts have been made to improve the quality of MDPM in order to allow its wider utilization in formulated meat products. These modifications are aimed at improving functional and physicochemical properties of MDPM. Several methods, including centrifugation, pH adjustment and salt preblending, pasteurization, use of LAB, mechanical mixing/thermal binding, use of vegetable proteins, extrusion and texturization, and surimi processing have been considered in order to achieve the above objectives.

Centrifugation of MDPM has been reported to improve emulsion characteristics, water retention capacity and flavour stability of the meat (Froning and Johnson, 1973;

Dhillon and Maurer, 1975a, 1975b). Partial removal of heme components and lipids are probably responsible for the improvement of flavour stability, and better emulsifying properties of products and these effects may be related to the higher protein and lower fat contents in the centrifuged meat fraction.

Studies have also been done on modification of MDPM through pH adjustment and/preblending with salts. These conditions enhanced emulsion stability of the prepared products (Froning and Janky, 1971). Use of salt preblending in conjunction with pH adjustment to improve the emulsifying ability could also be considered. pH adjustment of the meat from 5.0 to 8.0 increases binding of meat particles in broiler loaves (Maesso *et al.*, 1970a). Use of MDPM, preblended with salt, as a sausage component produced products with improved firmness and chewiness as compared with those using MDPM without salt preblending (Jantawat and Carpenter, 1989).

Pasteurization and addition of LAB have been reported to improve microbial stability of MDPM. Mast and MacNeil (1975) demonstrated that heat pasteurization greatly reduced bacterial levels of MDPM and hence its shelf-life. Raccach (1977) reported that lactic acid starter cultures, *Pediococcus cerevisae* and *Lactobacillus plantarum* repressed psychrotrophic bacterial population. The LAB were found to be effective inhibitors of three Pseudomonas species in cooked MDPM and lowered TBA values during storage at 3 °C for 7 days (Raccach and Baker, 1979).

Mechanical mixing was reported to promote the formation of sticky exudates,

which functioned as an excellent binder (Aref and Tape, 1968). Similar results were obtained by Maesso *et al.* (1970a,b). However, Schnell *et al.* (1970) stated that the binding of meat was a complex heat-mediated phenomenon involving a summation of water binding capacity and/or water holding capacity, protein solubility and cell disruption. It has also been reported that frankfurters containing up to 30% heated MDPM were satisfactory based on sensory and physical evaluation. However, incorporation of higher levels of MDPM in products resulted in inferior quality due to loss of emulsifying and binding ability of proteins denatured in the heating process (Young and Lyon, 1973). However, MDPM is used at 100% level in some meat products (Dawson *et al.*, 1989).

Research interest during the past two decades has been directed towards improving the functional properties of MDPM and to give products shape and form by mixing them with various textured soy protein ingredients or by using extrusion processing. Use of processed plant proteins in meat products is governed by existing regulations. Isolated soy protein (ISP) has good functional properties related to emulsification, fat-binding, water-absorption, adhesiveness and gelation. ISP could be used at a level of 2% in sausages in the United States (Pomeranz, 1985). Addition of ISP enhances shear force and resistance to chewing, dilutes pigments, improves moisture retention and reduces oxidation due to antioxidant effects of soy and/or lower fat content of the soy/meat blend as compared to products with no soy protein (Kumar and Wismer-Pedersen, 1983). Lyon

et al. (1981) observed that poultry rolls containing 1-2% isolated soy protein (ISP) and 10 or 20% MDPM exhibited higher cook yield than rolls with ISP but without MDPM. As the level of MDPM increased from 0 to 20%, rolls became darker, redder and more yellow, as indicated by objective colour measurements, however, the cook yield increased by only 2%.

Attempts have also been made to texturize MDPM which would offer advantages for use in a variety of fabricated products. Extrusion processing of proteins with heat and pressure is widely used. Acton (1973) carried out a study on texturization of MDPM by particulate binding of tissue using a dry-heat process. Water holding capacity of the texturized meat strands measured as the amount of retained 0.06M NaCl solution by the meat strands after centrifugation, increased as texturization time increased. In an earlier study, Acton (1972) found that binding strength development during processing of meat loaves correlated well with denaturation and loss of extractability of the salt-soluble proteins. Megard *et al.* (1985) investigated the restructuring of MDCM by high temperature short time (HTST) extrusion cooking. They concluded that it was possible to continuously compress and texturize mechanically deboned meat in twin-screw cooking extruders. Alvarez *et al.* (1990) also used a twin-screw cooking extruder to restructure MDCM in combination with three nonmeat binders: corn starch, ISP and wheat gluten, at concentrations of 10-30%. They found corn starch to be the best binder for the products which were evaluated. It was also reported that fat content and lipid oxidation

decreased as extrusion temperature increased.

2.15 Washing of minced meats and preparation of surimi-like products

Surimi is a Japanese term for mechanically deboned fish flesh that has been washed with water and mixed with cryoprotectants for long frozen shelf life (Lee, 1984). It is widely marketed as a highly functional protein ingredient used in the manufacture of a variety of seafood analogues (Lanier, 1986). However, the myofibrillar proteins in the washed mince will lose their functional properties rapidly once they are frozen, hence cryoprotectants are used to prevent muscle proteins from denaturation during frozen storage. The cryoprotectants generally include 4% sugar, 4-5% sorbitol and 0.2-0.3% polyphosphate (Babbitt, 1986). Washing plays an important role in removing undesirable matter such as blood, fat, and odorous substances and concentrates actomyosin, thus improving the gel-forming characteristics of surimi. Surimi is a wet concentrate of the myofibrillar proteins of fish which possesses enhanced gel-forming, water-holding, fat binding and other functional properties relative to minced fish (Okada, 1992). In the manufacturing of surimi-based products, the raw or frozen surimi is ground with salt and other ingredients, then extruded or composite-moulded depending upon the form of final product desired, and finally heat processed to set the shape, develop the texture, and pasteurize the product (Okada, 1992). Flavouring compounds are also added during processing in order to impact adequate taste to seafood analogue formulations. The

traditional Japanese kneaded foods called "Kamaboko" and imitation shrimp, scallop and crab meat products are made from surimi. The surimi technology has enabled the production of protein products from underutilized species such as Alaska pollock (Lee, 1984; Lanier, 1986; Pigott and Tucker, 1990).

Surimi production could be extended to MDPM in order to prepare products with low fat, low hemoprotein and a light-colour. Use of aqueous and saline solutions at various pH conditions in the washing of MDPM has been reported by various authors. Washed MDPM so obtained had a lighter colour and reduced amounts of lipid and pigment (Hernandez *et al.*, 1986; Dawson *et al.*, 1988; Lin and Chen, 1989; Shahidi *et al.*, 1992).

Ball and Montejano (1984) first reported the washing of MDPM with tap water. They also used 0.5% $NaHCO_3$ solution in the alkaline pH range to extract fat and pigments from MDPM. Hernandez *et al.* (1986) tested the washing of MDTM with 0.04M phosphate buffer solutions (pH 6.4-8.0) and then recovered and dewatered the washed meat on a cheese cloth. The washed MDTM was lighter and less red in colour as the pH of the washing solution increased. Phosphate buffer (pH 8.0) was most effective in removing the pigments. A similar method was reported by Elkhalifia *et al.* (1988) using 0.03M potassium phosphate buffer (pH 5.8, 7.4 and 8.0) and 0.02M sodium acetate buffer (pH 5.2) in washing turkey thigh tissues. The latter products were lighter in colour and had improved water holding capacity due to the removal of sarcoplasmic

proteins. The use of washing procedure involving $NaHCO_3$ solution was reported by Dawson *et al.* (1988, 1989) who investigated the washing of MDCM in a pilot-scale study. They reported that the procedure, with a protein yield of 15.8%, was effective in removing fat and pigments from MDCM. Incorporation of spray-dried egg white (SDEW) to washed MDCM enhanced moisture and protein content of the products as compared with its unwashed MDCM gels (Dawson *et al.*, 1990a). These researchers also reported an increase in cook yield and decreased deformability of the gels when 7.5% SDEW was incorporated.

In a more recent study, Dawson *et al.* (1990b) reported the changes in the neutral and phospholipid fractions of MDCM due to washing, cooking and storage. They observed that MDCM readily oxidized during storage at 4 °C. In particular, fatty acids of phosphatidylethanolamine (PE) and phosphatidylcholine (PC) + lysophosphatidylethanolamine (LPC) oxidized more readily than the other phospholipid fatty acids. Yang and Froning (1992) reported that washing of MDCM with 0.5% $NaHCO_3$ solution upon filtration and screen sieving (0.85 mm mesh) gave a yield of 18.7% of the original MDCM, on a dry weight basis. The meat captured on the screen contained 2.5 times more myofibrillar protein and 3.0 times more connective tissues than its unwashed counterparts. The washed meat that passed through the screen sieve contained 9.2 times more myofibrillar protein and 3.0 times less connective tissues than the unwashed MDCM, on a dry weight basis. However, none of these studies on aqueous

washing of MDPM has focused on possible loss of nutrients such as minerals and vitamins and flavour precursors in the washed MDPM.

OBJECTIVES OF THIS STUDY

The objectives of this study were: (1) To determine the most suitable aqueous washing medium that would effectively extract fat and heme pigments from MDCM. (2) To examine the effect of aqueous washing on yield, compositional and functional characteristics and colour quality of washed MDCM. (3) To assess the effect of washing on the removal of minerals and flavour precursors in MDCM. (4) To assess the effect of washing on the oxidative stability of MDCM.

CHAPTER 3

MATERIALS AND METHODS

3.1 Sample preparation

Samples of mechanically deboned chicken meat (MDCM) were prepared from 40 day-old birds (*Arbor acre*) after removing of skin from backs and necks, using a Poss deboner model PDE 500 (Poss Limited, Toronto, Ontario) at a setting of 8 (maximum-10). Removal of skin from poultry parts prior to mechanical deboning is routinely practised by several processors in Canada, including J.M Schneider Inc. (Horizon Poultry, (Kitchener, Ontario) and Newfoundland Farm Products Corp. (St. John's, Newfoundland). Other sources of MDCM were legs and backs, and breast and backs which were prepared as above. Preparation of MDCM was carried out on 6-12 h *post mortem* carcass portions at the Newfoundland Farm Products Corporation and J.M. Schneider Inc., (Horizon Poultry, Kitchener, Ontario). The hand deboned chicken meat (HDCM) from breasts, necks and backs, and legs and backs were ground twice using a Braun Multipac meat grinder (Braun Inc., Frankfurt, Germany) with 3 mm holes size plate. Samples were then stored at -20 °C for up to 2 wk and then thawed at +4 °C for 12 h before use.

Samples of MDCM prepared from necks and backs were washed two times. The first washing used water and the pH of the meat-water mixture was 6.9; the second washing used water (pH of mixture 5.2 or 7.2) or a 0.5% solution of either NaCl (pH=6.9) or $NaHCO_3$ (pH=7.8). In another set of experiments, the pH of the meat-water

mixture was adjusted to 5.2 using a 5% (v/v) acetic acid solution. Each washing was carried out at 2 °C for 10 min while stirring manually. The ratio of the extraction solution to meat was 3:1 (v/w) unless otherwise specified. Washed meats were then filtered through three layers of cheese cloth with 1 mm size holes. Each washing was replicated three times using MDCM from meat prepared on the same and different days. These samples were used for analyses. Since there were small and non-significant variations in data obtained on different days, the data of only one sample was reported. MDCM from necks and backs was used in all experiments. For the oxidative stability studies, other types of MDCM and HDCM were used for comparison.

3.2 pH measurement

pH measurements were carried out using a Fisher Accumet pH meter model 805MP (Fisher Scientific Co., Montreal, Quebec) by immersing a combination electrode into the meat-solution mixture (1:3, w/v) or solution whose pH was to be determined.

3.3 Proximate composition

3.3.1 Moisture content

Approximately 3-4 g of MDCM was accurately weighed into a preweighed aluminium dish and placed in a forced-air convection oven (Fisher Isotemp 300, Fair Lawn, New Jersey) which was preheated to 105±1 °C. Samples were held at this

temperature overnight or until a constant weight was obtained. The moisture content was calculated as the percent ratio of the weight difference of the samples before and after drying to that of the original material (AOAC, 1990).

3.3.2 Crude protein

Approximately 0.3-0.4 g of sample was accurately weighed on a nitrogen-free paper and placed in a digestion tube of a Büchi 430 digestor. The nitrogen content in different samples was determined by digestion in 20 mL of concentrated H_2SO_4 in the presence of two Kjeltab catalyst tablets (Profamo, Dorval, Quebec) in the digestor until a clear solution was obtained. Digested samples were diluted with 50 mL of distilled water followed by addition of 150 mL of a 25% (w/v) NaOH solution. Nitrogen in the samples was converted to ammonia which was steam-distilled (Büchi 321) into a 4% (w/v) H_3BO_3 solution (50 mL) containing a few drops of end point indicator (EM Science, Gibbstown, New Jersey). Approximately 200 mL of distillate was collected. The content of ammonia in the distillate was determined by titrating it against a 0.1N standardized H_2SO_4 solution (AOAC, 1990). The crude protein content in the sample was calculated as %N x 6.25.

3.3.3 Determination of total lipids

Total lipids were extracted with a mixture of chloroform and methanol as described by Bligh and Dyer (1959). Approximately 25 g of sample was accurately weighed and then extracted with a mixture of 25 mL chloroform and 50 mL methanol (1:2 v/v) by homogenizing for 3 min with a Polytron homogenizer (Brinkmann Instruments, Rexdale, Ontario) at speed level 4. A further extraction was done with the addition of 25 mL chloroform followed by homogenization. About 25 mL of distilled water was added and the mixture was then filtered through a Buchner funnel using a Whatman filter paper No 3 (Fisher Scientific Co.). The filtrate was separated overnight in a separatory funnel. Dilution with chloroform and water resulted in separation of homogenate layers and inclusion of lipids in the chloroform. A 10 mL aliquot of the lipid extract in chloroform, after drying over anhydrous sodium sulphate, was transferred into a tared 50 mL round bottom flask and the solvent was removed under vacuum using a Büchi RE 111 rotavapor (Büchi Laboratoriums, Switzerland). The flask was then placed in a forced-air convection oven at 80 °C for 1 h. After cooling in a desiccator, the lipid content was determined gravimetrically.

3.3.4 Ash content

Approximately 3-4 g of sample was weighed into a cleaned porcelain crucible and then charred over a Bunsen burner and subsequently placed in a temperature controlled

muffle furnace (Blue M Electric Co., Blue Island, Illinois) which was preheated to 550 °C. Samples were held at this temperature until a grey ash was produced and then cooled in a desiccator and weighed immediately. Ash content was calculated as percent ratio of the weight of the ash obtained after ignition to that of the original material (AOAC, 1990).

3.4 Content of hemoproteins

The total hemoprotein pigments were determined as their hemin equivalents according to the method of Hornsey (1956). About 4 g of sample was homogenized with 20 mL acetone/water/concentrated HCl 8.0:1.1:0.2 (v/v/v), for 2 min using a Brinkmann Polytron homogenizer (speed level 4). The homogenate was centrifuged for 10 min at 2000 x g using an IEC clinical centrifuge (Fisher Scientific Co.), and the residue was further homogenized in 20 mL acetone/water/concentrated HCl 40:9:1 (v/v/v) for 1 min. Supernatants from both centrifugations were combined and incubated in the dark for 1 h. The solution was filtered through Whatman No. 3 filter paper and diluted to 100 mL with the latter solvent mixture. Absorbance (A) was read at 640 nm against a blank of acetone/water/HCl, using a Hewlett Packard diode array spectrophotometer model 8452A (Montreal, Quebec). Total hemin content in 10 g of meat extracted into 50 mL of 80% acetone was calculated as A_{640} x 680, where 680 is a factor derived from the extinction coefficient of acid hematin in 80% acetone (Hornsey, 1956). The factor 680 was

corrected when a different amount of meat or extraction solution was employed. The total hemoprotein pigments was calculated from hemin content.

Total hemoprotein pigments (mg/g sample)

$$= [\text{Hemin content (mg/g sample)}/MW_{hemin}] \times MW_{myoglobin}$$

where: $MW_{myoglobin} = 17{,}500$

$MW_{hemin} = 616.5$

The content of myoglobin (Mb) and hemoglobin (Hb) in MDCM was determined according to Warris (1976). Hemoprotein pigments were extracted from 20 g of MDCM in 185 mL of ice-cold 0.04M phosphate buffer at pH 6.8 by homogenizing the sample with a Polytron homogenizer (speed level 4) for 75 s. The extract was incubated for 1 h at 4 °C, followed by centrifugation at 2000 x g for 15 min using an IEC clinical centrifuge. The extract was filtered through Whatman No. 3 filter paper and a few mg of potassium ferricyanide (Fisher Scientific Co.) were added to convert the pigments to their more stable cyanomet derivatives. About 10 mL of the extract was set aside for determination of total pigment concentration and the remaining solution was frozen and kept at -20 °C. The frozen extracts were allowed to thaw at 4 °C until about 15 mL of liquid was formed. The extracts, both the original unconcentrated sample and the preconcentrated solution, were then clarified by centrifugation at 30,000 x g for 60 min using a Sorval superspeed RC2-B centrifuge (Du Pont, Markham, Ontario). The absorbance of the clarified original extract was read at 540 nm using a Hewlett Packard

diode array spectrophotometer. The total hemoprotein pigment was calculated as A_{540} x 17,500 x 200/11,300 x 20 mg/g sample. This formula uses extinction coefficient and Mw for Mb given by Drabkin (1950) and Low and Rich (1973), respectively (Warris, 1976).

The preconcentrated extract was freeze dried, redissolved in 1 mL of water and dialysed against 0.5M NaCl solution to precipitate other sarcoplasmic proteins which were removed by centrifugation at 2000 x g using an IEC clinical centrifuge. Hb and Mb were then separated from each other by applying 1 mL of this extract to a 30 cm x 1 cm i.d. column packed with Sephadex G-75 having a particle size distribution of 40-120 μ (Pharmacia Fine Chemicals, Uppsala, Sweden). The eluent buffer was 0.1M mono- and dibasic phosphate mixture at pH 6.8, containing 0.1M NaCl (Fisher Scientific Co.). The absorbance (A) of the Hb fraction which was almost completely excluded from the gel, was read at λ = 420 nm and that of Mb which was more slowly moving band, at λ = 540 nm using a Hewlett Packard diode array spectrophotometer. The ratio of cyanomethemoglobin at 540 nm to that at 420 nm was 0.11. The amount of Mb was calculated using the equations given below (Warris, 1976).

Mb in extract (%) = $A_{540}/(A_{420} \times 0.11) + A_{540}$

Mb (mg/g sample) = [Total hemoprotein pigments(mg/g sample) x Mb in extract (%)]/100

The Hb content was calculated as the difference between total pigment and Mb contents.

3.5 Tristimulus colour parameters

The tristimulus colour parameters, namely Hunter **L** (100, white; 0, black), **a** (+, red; -, green) and **b** (+, yellow ; -, blue) values of the samples were determined by surface reflectance measurements using a Colormet colourimeter (Instrumar Engineering Limited, St. John's, Newfoundland). The unit was standardized with a B-143 white calibration tile. Its Hunter values were **L**, 94.5 ± 0.2; **a**, -1.0 ± 0.1; and **b**, 0.0 ± 0.2. Measurements were made on a layer of MDCM, 25 mm thick, in a sealed 23 cm x 17 cm transparent polythene pouch. Generally 6 readings were recorded at different areas of the meat surface.

3.6 Amino acid composition and protein efficiency ratio (PER)

Samples were lyophilized, ground to fine powders, and immediately analyzed for their amino acid composition. For the determination of amino acids, powders were digested in 6N HCl at 110 °C under a stream of nitrogen for 24 h (Blackburn, 1978). The amino acid composition of the hydrolysates was determined using a Beckman 121MB Amino Acid Analyzer (Beckman, Palo Alto, California) and the three buffer sodium citrate method. Cysteine and methionine were first oxidized in a performic acid solution followed by their hydrolysis in 6N HCl and were determined as cysteic acid and methionine sulphone, respectively (Blackburn, 1978). Performic acid was prepared by adding 1 mL of 30% H_2O_2 (Fisher Scientific Co.) to 9 mL of 88% CHCOOH (Fisher

Scientific Co.), allowed to stand for 1 h at room temperature, then cooled to 0 °C. Analysis of tryptophan was performed by UV absorption after hydrolysis of the sample in 3N mercaptoethane sulphonic acid (Pierce, Rockford, Illinois) at 110 °C under vacuum as described by Penke *et al.* (1974). Connective tissue content was calculated as 8.03 x hydroxyproline content (Karatzas and Zarkadas, 1988).

The protein efficiency ratio (PER) was calculated by the amino acid scoring methods developed by Lee *et al.* (1978) and given as follows:

$$PER = 0.08084\ \Sigma AA_7 - 0.1094 \qquad (1)$$

$$PER = 0.06320\ \Sigma AA_{10} - 0.1539 \qquad (2)$$

Where ΣAA_7 = Isoleucine + Leucine + Lysine + Methionine + Phenylalanine + Threonine + Valine; and $\Sigma AA_{10} = \Sigma AA_7$ + Arginine + Histidine + Tryptophan.

3.7 Free amino acids and peptides

The content of free amino acids as well as carnosine and anserine (imidazole peptides) was determined by homogenizing 10 g of sample with 20 mL ice-cold 6% (w/v) perchloric acid with a Brinkmann Polytron homogenizer (speed level 4). After 30 min incubation in ice, samples were centrifuged using an IEC clinical centrifuge at 3000 x g for 10 min at 5 °C. The procedure was repeated and supernatants were combined. The pH of the supernatant was adjusted to 7.0 using a 33% (w/v) KOH solution. Perchlorate

salt was precipitated and removed after centrifugation for 10 min at 3000 x g. The supernatant was acidified with 10N HCl to pH 2.2, diluted to 50 mL with distilled water and then diluted at a 2:1 (v/v) ratio with a 0.3N lithium citrate buffer, pH 2.2. The extract was then analyzed on a Beckman 121MB Amino Acid Analyzer using Benson D-X 8.25 resin (Benson Company, Reno, Nevada) and a single column according to the three buffer lithium procedure as per Beckman 121MB application notes (Beckman 121MB-TB-017 application notes, 1979).

3.8 Determination of nucleotides

Approximately 10 g of sample was weighed and homogenized in 20 mL ice-cold 0.6N perchloric acid for 30 sec using a Brinkmann Polytron homogenizer (speed level 4). The homogenate was filtered through Whatman No. 1 filter paper, and the resultant filtrate, i.e., the nucleotides extract, was stored at -60 °C until use.

The nucleotide extract was thawed at 0-4 °C and the pH was adjusted to 6.5 by diluting it with 0.1M K_2HPO_4 at a ratio of 1:10 (v/v), in order to avoid crystallization of perchloric acid during HPLC analysis. This diluted extract was then filtered through a 0.45 µm nylon filter (Cameo II, MSI, Westboro, Massachusetts) into the HPLC sampling vial and was then used directly for HPLC analysis. Nucleotides in the extract were determined by a reversed-phase HPLC procedure (Schimadzu Corp., Kyoto, Japan). The column used was a 10 µm particle size LC-18-T reversed phase analytical column (4.5

mm x 24 cm, Supelco, Oakville, Ontario). An LC-18-T guard column (4.5 mm x 5 cm) was coupled with the analytical column. Twenty µL of a standard or filtered solution was injected into the column using an SIL-6B Auto-injector (Supelco). The detector response of each nucleotide and nucleoside was calibrated by injecting a known amount of individual reference compounds (Sigma Chemical Co.). A modified procedure according to Stocchi (Stocchi *et al.*, 1987) was used for the analytical separation conditions. These chromatographic conditions were: 0.01 to 3.8 min at 100% of buffer B, 7.8 to 15.0 min at 0 to 20% of buffer A, 15.0 to 19.5 min at 20 to 40% of buffer A, 19.5 to 27.0 min at 40 to 100% of buffer A. The gradient was uniform in each case and then immediately returned to 100% of buffer B and held until completion, 32 min. Buffer B was 0.1M potassium phosphate buffer, pH 6.0, containing 8 mM tetrabutylammonium hydrogen sulphate (Sigma Chemical Co.) and buffer A was 0.1M potassium phosphate buffer, pH 6.0, containing 8 mM tetrabutylammonium hydrogen sulphate and 30% methanol. The flow rate was 1 mL/min and detection was measured at 254 nm using Smimadzu SPD-6AV UV-VIS spectrophotometric detector. The analysis was performed at room temperature, but the samples were kept at 0 °C until injection. Quantification of the endogenous nucleotide and nucleoside concentrations was carried out using the data module in the external standardization mode.

3.9 Mineral content

The analysis of minerals was carried out using the Inductively Coupled Plasma (ICP) emission spectroscopic method (AOAC, 1990). Ashing was done by drying appropriate sample weights in an oven overnight at 100 °C, and then placing them in a 525 °C muffle furnace until white ash was formed (usually 5-8 h). The resultant ash was digested in 30 mL $HNO_3/HClO_4$ (2:1, v/v) in Kjeldahl flasks until the reaction of sample with $HClO_4$ was complete as identified by a cessation of effervescence between the sample and $HClO_4$. Digested samples were transferred to 50 mL volumetric flasks and diluted to volume with distilled water. Elemental determinations were done for us with the ICP emission spectrometer (Model 975 Plasma Atom Comp, Thermo Jarrell-Ash Corporation, Franklin, Massachusetts) by the Diversified Research Laboratories (Toronto, Ontario). Calibration of the instrument was done using known calibration standards. Computer calculation of the concentration of each element in each diluted solution was done and converted to its concentration in the original sample.

3.10 Content of nucleic acids

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were extracted according to the method of Schmidt-Thannhauser (1945) as modified by Munro and Fleck (1969). Five g of sample were homogenized in 80 mL ice-cold deionized water using a Polytron homogenizer (speed level 4). Five mL of the homogenate was allowed to stand

in ice for 10 min and then centrifuged at 2000 x g for 10 min using an IEC clinical centrifuge. The residue was washed with 2.5 mL of ice-cold 0.2N perchloric acid, then centrifuged at 2000 x g for 10 min. The residue obtained was digested in 4 mL of 0.3N KOH for 1 h at 37 °C in a water bath. The resultant solution was cooled in ice, and 2.5 mL of 1.2N perchloric acid was added to it, then allowed to stand for 10 min, during which time proteins precipitated. The mixture was centrifuged at 2000 x g for 10 min and the supernatant recovered as extract No. (1). The precipitate was washed twice with 2.5 mL of 0.2N perchloric acid, centrifuged at 2000 x g for 5 min, the precipitate was dissolved in 17 mL of 0.3N KOH at 37 °C, then diluted with distilled water to the 50 mL mark. This solution was used for determination of DNA. The supernatant obtained was combined with extract No. (1) and 10 mL of 0.6N perchloric acid was added, then diluted with distilled water to 100 mL. This solution was used for RNA determination.

DNA in the samples was estimated by determining the deoxyribose content in the extract using the indole procedure of Ceriotti (1952). RNA was determined by an ultraviolet spectrophotometric procedure by recording the absorbance values of nucleotide extracts at 260 nm using a Hewlett Packard diode array spectrophotometer. Protein interference at this wavelength was eliminated by applying a correction factor of 0.001 absorbance unit per 1 µg/mL protein concentration in the extracts. The Folin-phenol procedure of Lowry *et al.* (1951) was used to measure protein concentrations. Bovine serum albumin (Sigma Chemical Co.) was used for standardization (Appendix A.3). Calf

Thymus DNA (containing 82% single-stranded DNA) and Calf liver RNA (96% purity, both Sigma Chemical Co.) were subjected to the same treatments and were used as standards (Appendix A.1 and A.2).

3.11 Cholesterol content

The cholesterol content in the raw and cooked samples was determined according to Rudel and Morris (1973) using o-phthalaldehyde reagent (Sigma Chemical Company, St. Louis, Missouri). The MDCM was cooked in sealed pouches before being used for the cooked MDCM analysis. A 0.2 mL chloroform extract of the total lipids obtained according to Bligh and Dyer (1959) was saponified in 0.3 mL of a 33% (w/v) KOH solution (Fisher Scientific Co.) in the presence of 3 mL of 95% ethanol in 20 mL screw capped tubes and mixed thoroughly. Tubes were then heated to 60 °C in a water bath for 15 min, cooled and mixed with 10 mL hexane and 1 mL of distilled water. Tubes were capped and mixed thoroughly using a Fisher Vortex mixer (Fisher Scientific Co.). A blank, a standard and samples of chloroform extract from MDCM were extracted at the same time. Appropriate 1 mL aliquots of the hexane layer were pipetted into clean tubes and the solvent was then evaporated under a stream of nitrogen. Two mL of the 0.05% (w/v) o-phthalaldehyde reagent in glacial acetic acid and 1 mL of concentrated H_2SO_4 were carefully added to the tube and then mixed thoroughly. The absorbance of the solutions was read at 550 nm using Hewlett Packard diode array spectrophotometer. A

standard curve was prepared using a cholesterol solution (Fisher Scientific Co.) at concentrations ranging from 1.5 to 14.0 µg/mL (Appendix A.4).

3.12 Determination of the content of sulphydryl groups and disulphide bonds contents, protein solubility and degree of thermal coagulation

3.12.1 Sulphydryl groups and disulphide bonds

Free sulphydryl groups (SH) were determined in 0.10 to 0.15 g of meat sample dissolved in 8 mL of 0.75% ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and 0.035M sodium dodecyl sulphate (SDS) solution from Sigma Chemical Co. in a 0.2M Tris buffer (Sigma Chemical Co.) pH 8.2, according to the procedure given by Opstvedt *et al.* (1984). After standing for 2 h, 0.5 mL of a 0.016M solution of 5,5′-dithiobis(2-nitrobenzoic acid) (DNTB) (Sigma Chemical Co.) in methanol and 31.5 mL of methanol were added with mixing. The solution was allowed to stand at 20 °C for 15 min, centrifuged at 3000 x g for 15 min using an IEC clinical centrifuge, and the absorbance was read at 412 nm according to Ellman (1959) using a Hewlett Packard diode array spectrophotometer. Hydrolysis of DTNB was accounted for in a no protein blank. A calibration curve for sulphydryl groups was prepared using a reduced glutathione solution (Sigma Chemical Co.) in concentrations ranging from 0 to 0.1 mg/mL of sample (Appendix A.5).

Disulphide bonds were determined as follows: meat samples containing about 35

mg of protein were dissolved in 4 mL of 0.15M 2-mercaptoethanol solution (Sigma Chemical Co.) in 8M urea (Fisher Scientific Co.). The disulphide bonds were then calculated from the difference between the content of SH in the reduced and in the original samples as: Disulphide bonds = (Total reduced SH - Free SH x 0.97)/2 (Synowiecki and Sikorski, 1988).

3.12.2 Protein solubility and degree of thermal coagulation

For determination of protein solubility, 5 g of meat was homogenized for 1 min, using a Polytron homogenizer (speed level 4), with 100 mL of 5% (w/v) NaCl solution in 0.003M $NaHCO_3$, 0.035M SDS in 0.003M $NaHCO_3$ at pH 7.0 while in an ice bath. After 30 min of solubilization of the meat sample with intermittent mixing using a Fisher Vortex mixer and 10 min centrifugation at 10,000 x g using a Sorval Superspeed RC2-B centrifuge, the total protein in the supernatant was determined by the Kjeldahl method (AOAC, 1990). The degree of thermal coagulation measured as a loss of solubility was determined by heating the MDCM extracts in a 5% (w/v) NaCl solution in 0.003M $NaHCO_3$ for 40 min at 40, 50, 60, 75 and 99 °C in a water bath. The degree of thermal coagulation was expressed as $(c_1 - c_2) / c_1 \times 100$, where c_1 and c_2 are the concentrations of proteins before and after heat treatment, respectively.

3.13 Functional properties

3.13.1 Cook yield

A 10 g sample was transferred into a pre-weighed centrifuge tube along with 5 g of water. Tubes were covered with aluminium foil and then placed into a boiling water bath for 20 min. The tubes were cooled to room temperature and then centrifuged for 15 min using an IEC clinical centrifuge at 3000 x g. The juice released was decanted and the sample was blotted on a Whatman No. 1 filter paper and transferred back into the tube. The percent cook yield was calculated from the weight difference data.

3.13.3 Emulsion stability

The method of Townsend *et al.* (1968) was used to determine the emulsion stability of samples. A 15 g sample was blended with 45 mL of 3% (w/v) NaCl solution for 1 min using a Brinkmann Polytron homogenizer (speed level 4). A 25 g corn oil sample was added to the mixture over a 5 min period while blending and further blending for 1 min after all oil was added. Duplicate 35 g emulsion samples with known volumes were stuffed in 50 ml graduated centrifuge tubes, covered with aluminium foil and placed in a water bath at 85 °C for 15 min. The tubes were cooled under running tap water for 15 min. Centrifugation was repeated until the volume of fluid remained constant. Emulsion stability was expressed as percent stable emulsion formed with respect to the total volume of the initial emulsion.

3.13.2 Emulsifying capacity

A method similar to that of Swift *et al.* (1961) was used for determination of the emulsifying capacity of each sample. A 50 g sample was blended with 200 mL of a cold 3% NaCl solution using a Polytron homogenizer (speed level 3) in an Erlenmeyer flask for 2 min. Fifteen g of the resulting slurry was placed in a clean 600 mL beaker with 37.5 mL of 3% (w/v) NaCl solution and mixed for 5 s. Then 50 mL of corn oil was added directly to the meat slurry and mixing was resumed while additional oil was added continuously from a burette at a rate of 1 mL per s. Addition of oil was terminated when the emulsion collapsed which coincided with an increase in the audible pitch of the blending motor. Emulsifying capacity of meat was reported as mL of oil emulsified by 2.5 g of meat.

3.14 Separation of polar and nonpolar lipids and analysis of fatty acid composition

Extraction of total lipids from the MDCM was done according to Bligh and Dyer (1959). Silicic acid column chromatography was used to separate the neutral and polar lipids. About 100 g of activated silicic acid (Mallincrodt, 100 mesh, Pointe-Claire, Quebec) was mixed with 10 column volumes of chloroform and packed into a 35cm x 2.3 cm i.d. glass column. The column was washed several times with chloroform prior to use. About 1.25 g lipid sample was dissolved in a small amount of chloroform and was then applied to the top of the packed column. Two classes of lipids were eluted from

the column, using in sequence 800 mL each of chloroform and methanol. Neutral lipids were eluted from the column with chloroform while the methanol eluent contained polar lipids, predominantly phospholipids. Solvents were then evaporated under reduced pressure at 40 °C using a Rotavapor RE 111. Traces of solvent were removed by a stream of nitrogen.

About 100 mg of phospholipids obtained from the silicic acid column separation was dissolved in 1 mL chloroform/water (2:1, v/v) and applied onto a 20 cm x 20 cm precoated layer of 250 µm thick silica Gel G plate (Sigma Chemical Co.) using a capillary pipette. The plates were developed in a chamber containing chloroform/methanol/water (25:10:1, v/v/v). Spots were visualized under UV light. Identifications were made by comparing the R_f values of bands with those of known standards (Sigma Chemical Co.). Spots were scrapped from TLC plates and subjected to phosphorous content determination. Components were eluted with chloroform/methanol/water (5:5:1, v/v/v). Phospholipids were digested in 70% (w/v) perchloric acid with release of inorganic phosphate which was reacted with 5% (w/v) ammonium molybdate (Sigma Chemical Co.) to form phosphomolybdic acid (Christie, 1982) which was subsequently reduced by using ascorbic acid. The solution was evaporated to dryness and to this digest, 5 mL of distilled water, 3 mL of 1.5N H_2SO_4 and 0.4 mL of 2% ascorbic acid (Fisher Scientific Co.) were added and mixed. The solution was allowed to stand for 20 min and its absorbance was measured at 660 nm using a Hewlett Packard diode array

spectrophotometer. Two millimetres of KH_2PO_4 solution containing 3.2 x 10^{-3} mg of phosphorus was used as working standard for the phosphorus determination (Nahapetian and Bassiri, 1975).

The total lipids, neutral lipids and phospholipids were hydrolysed and converted to methyl esters by transmethylation in 6% (v/v) H_2SO_4 in 99.0 mole% pure methanol (BDH Inc., Ville St-Laurent, Quebec) at 65-70 °C for 15 h (Keough and Karil, 1987). After extraction of the methyl esters into hexane, esters were analyzed using a Perkin-Elmer 8310 gas chromatograph (Hewlett Packard, Toronto, Ontario) equipped with a 30 m x 0.25 mm column (SP 2330, Supelco Inc.). Oven temperature was initially 180 °C for 12 min and was ramped to 200 °C at 20 °C/min and held there for 8 min. The injection port and flame ionization detector temperatures were 230 °C. The flow rate of the helium carrier gas was 25 mL/min. Identification of fatty acid methyl esters was based on the comparison of their retention times with standards (Supelco Inc.). Quantification was performed by computer calculation of the instrument using area normalization. Results were presented as area% of fatty acids relative to the total fatty acids.

3.15 Thiobarbituric acid (TBA) test

The distillation method of Tarladgis *et al.* (1964) was adapted in this work. In all cases 10 g of meat was placed into a 500 mL round-bottom flask containing 97.5 mL

distilled water and 2.5 mL 4N HCl, along with a few drops of Dow Antifoam A (Sigma Chemical Co.) and several glass beads. The mixture was then heated for approximately 20 min to collect 50 mL of distillate.

The distillate was shaken thoroughly and 5 mL of it was pipetted into a 50 mL vial containing 5 mL 0.02M aqueous solution of 2-thiobarbituric acid (Sigma Chemical Co.). The vial was capped and heated in a boiling water bath for approximately 40 min to obtain a pink-coloured solution. After cooling to room temperature, the absorbance of the resultant complex was read at 532 nm using a Hewlett Packard diode array spectrophotometer. The compound 1,1,3,3-tetramethoxypropane (Sigma Chemical Co.) was used as a standard and TBA values (mg malonaldehyde equivalent/kg meat) were calculated using the equation of the standard line (Appendix A.6).

3.16 Statistical analysis

Analysis of variance and Tukey's Studentized Range Test (Snedecor and Cochran, 1980) were used to determine differences in mean values based on data collected from 3-6 replications of each measurement on Statistical Analysis System (SAS Inc., 1990, Cary, North Carolina). Significance was determined at a 95% level of probability.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Yield and proximate composition

The effect of various washings on the recovery of protein in meat samples is summarized in Table 4.1. Protein recovery after the first washing with water at pH 6.9 was 58.1%; while after the second aqueous washing at pH 7.1, it decreased to 52.6%. These values are somewhat higher than those reported by Dawson *et al.* (1988) for washed MDPM held on a steel screen. This is perhaps due to a difference in the size of the filter pores which was 3.9 mm in their study as compared to that of 1 mm in this work. The second washing of MDCM with salt or water at pH 5.2 also decreased the amount of recovered protein to 53.8 and 54.1%, respectively. Washing of meat with $NaHCO_3$ solution at pH 7.8, farthest from the isoelectric point of myofibrillar proteins (typically 5.2-5.5) gave the lowest protein yield of 41.7%.

All washings brought about a higher moisture and a lower fat content in the resultant samples as compared with those of their unwashed counterparts (Table 4.2). Moisture contents after the first and second washings with water at pH 6.9 and 7.1 were increased by 12.5 and 14.8%, respectively, over the moisture content of 73.9% in the unwashed meat. The lowest moisture increase was for samples washed with water at pH 5.2. A lesser degree of hydration of protein molecules occurs at a pH near their isoelectric point.

The crude protein content in unwashed MDCM was reduced from 14.3% to 11.4% after the first washing with water. The apparent decrease in protein content was due to

Table 4.1 Percent recovery of MDCM and protein after aqueous washings[1].

Treatment	pH	Dry weight of recovered meat (%)	Recovered protein (%)[2]
Unwashed MDCM	6.8	100[a]	100[a]
Washed			
1 x H_2O	6.9	51.2±0.8[b]	58.1±1.4[b]
2 x H_2O	7.1	40.3±0.6[d]	52.6±1.3[c]
Washed once with H_2O, and then			
0.5% NaCl	6.7	41.9±0.6[d]	53.8±1.0[c]
0.5% $NaHCO_3$	7.8	40.3±0.5[d]	41.7±0.9[d]
H_2O at pH 5.2	5.2	45.4±0.4[c]	54.1±0.6[c]

[1]Results are mean values ± standard deviation of 3 separate washings of the same meat sample. Values in each column with the same superscript are not significantly ($p>0.05$) different from one another.

[2]Recovered protein (g) = Recovered meat (g) x (% protein/100).

Table 4.2 Percent moisture and crude protein content of unwashed and washed MDCM[1].

Treatment	Moisture (%)	Crude protein, N x 6.25	
		Wet basis (%)	Dry basis (%)
Unwashed MDCM	73.9±0.2[d]	14.3±0.3[a]	54.8±0.7[d]
Washed			
1 x H_2O	83.1±0.1[b]	11.4±0.4[b]	67.5±0.4[a]
2 x H_2O	84.8±0.5[a]	10.3±0.3[c]	67.6±0.4[a]
Washed once with H_2O and then			
0.5% NaCl	83.7±0.4[b]	10.2±0.1[c]	62.6±0.4[b]
0.5% $NaHCO_3$	85.9±0.3[a]	8.6±0.2[d]	61.0±0.3[c]
H_2O at pH 5.2	79.9±0.3[c]	12.0±0.2[b]	59.7±0.3[c]

[1]Results are mean values of 4 replicates ± standard deviation. Values in each column with the same superscript are not signficantly ($p>0.05$) different from one another.

a higher moisture content in washed meat as well as loss of sarcoplasmic proteins (Yang and Froning, 1992) during washing. On a dry weight basis, however, the protein content increased from 54.8% to 67.5% and 67.6% after the first and second washings with water (at pH 6.9 and 7.1, respectively). The protein content increased even though the sarcoplasmic proteins were removed during washings. This may be due to the fact that some lipids and water-soluble minerals were extracted from the meat. Adu *et al.* (1983) and Dawson *et al.* (1988) observed a similar trend when they washed rockfish flesh and MDPM, respectively, with water.

A large reduction in lipid content of all washed meat samples was observed. Part of the fat floated to the top during the extraction process and was subsequently skimmed off. Unwashed MDCM contained, on a dry basis, 38.6% fat and aqueous washing removed about 26% of this amount (Table 4.3). Similar results were obtained by Dawson *et al.* (1988) who reported the removal of 20.5-46.6% of lipids from the samples during aqueous washing of MDCM. The second washing with water, salt or bicarbonate solutions was not as effective in removing additional lipids from MDCM samples.

The ash content in the samples washed with various media was also lowered. The first and second washings with water reduced the ash content from 4.2% in the unwashed samples to 3.0% and 2.7% (or 3.0% at pH 5.2), on a dry basis, respectively (Table 4.3). However, meat washed with aqueous solution containing NaCl or $NaHCO_3$ exhibited less decrease in ash content due to the addition of minerals from these washing media.

Table 4.3 Percent total lipid and ash content of unwashed and washed MDCM[1].

Treatment	Total lipid		Ash	
	Wet basis	Dry basis	Wet basis	Dry basis
Unwashed MDCM	10.1±0.0^{a}	38.6±0.3^{a}	1.1±0.0^{a}	4.2±0.0^{a}
Washed				
1 x H_2O	4.8±0.1^{d}	28.4±0.2^{d}	0.5±0.0^{c}	3.0±0.0^{cd}
2 x H_2O	4.3±0.0^{e}	28.5±0.2^{d}	0.4±0.0^{d}	2.7±0.1^{d}
Washed once with H_2O and then				
0.5% NaCl	5.3±0.0^{c}	32.6±0.3^{c}	0.6±0.0^{b}	3.6±0.1^{b}
0.5% $NaHCO_3$	4.9±0.0^{d}	34.5±0.3^{b}	0.5±0.0^{bc}	3.8±0.1^{b}
H_2O at pH 5.2	7.1±0.0^{b}	35.1±0.3^{b}	0.6±0.0^{b}	3.0±0.1^{c}

[1]Results are mean values of 4 replicates ± standard deviation. Values in each column with the same superscript are not significantly ($p>0.05$) different from one another.

4.2 Hemoprotein content and meat colour

A large proportion of hemoprotein pigments present in the proteins of MDCM were removed as a result of aqueous washing (Table 4.4). The hemoproteins of the unwashed MDCM consisted of 3.42 ± 0.06 mg/g Hb and 1.06 ± 0.03 mg/g Mb. These values are similar to those reported by Froning and Johnson (1973) for mechanically deboned fowl meat which contained about 3.7 mg total pigments and 0.7 mg Mb/g. The total content of hemoprotein pigments in MDCM after the first and second washings with water at pH 6.9 and 7.1 decreased by 39.9% and 47.3%, respectively. However, washing with water and then 0.5% $NaHCO_3$ solution was the most effective in removing hemoproteins from MDCM and their content decreased by 73.9%. Mb and Hb are water-soluble proteins with isoelectric points of 7.0 and 6.9, respectively. The presence of $NaHCO_3$ raised the pH of the meat slurry to 7.8, which enhanced the solubility of the pigments and this may have facilitated their removal. The higher pH may also result in a more open myofibrillar protein structure, which allows easier extraction of Mb (Dawson *et al.*, 1989). Hernandez *et al.* (1986) obtained similar results using aqueous solutions at pH 8.0 as compared with those at pH values of 6.4, 6.8 or 7.2.

The effect of aqueous washings on the colour of MDCM as assessed by Hunter colour values is also presented in Table 4.4. The first washing of MDCM with water increased the Hunter **L** value and decreased the **a** value of the product by 10.7% and 16.6% respectively. However, a second washing with water had little effect on Hunter

Table 4.4 Influence of the total content of hemoproteins on Hunter **L**, **a** and **b** values of MDCM[1]

Treatment	Total hemoproteins (mg/g)	Hunter values		
		L	**a**	**b**
Unwashed MDCM	4.48±0.19[a]	49.5±0.3[d]	22.2±0.6[a]	13.3±0.3[a]
Washed				
1 x H_2O	2.69±0.02[b]	54.8±0.3[c]	18.5±0.4[b]	13.6±0.3[a]
2 x H_2O	2.36±0.05[c]	55.5±0.6[c]	17.7±0.3[b]	13.4±0.4[a]
Washed once with H_2O and then				
0.5% NaCl	1.85±0.01[d]	57.2±0.3[b]	15.4±0.3[c]	13.9±0.3[a]
0.5% $NaHCO_3$	1.17±0.04[e]	61.5±0.5[a]	12.1±0.3[d]	13.3±0.4[a]
H_2O at pH 5.2	2.03±0.03[d]	56.8±0.4[b]	16.0±0.2[c]	14.3±0.3[a]

[1]Results are mean values of 4 replicates ± standard deviation. Values in each column with the same superscript are not significantly ($p>0.05$) different from one another.

L and **a** values. MDCM after washing with 0.5% $NaHCO_3$ solution at pH 7.8 gave higher **L** and smaller **a** values than samples washed with water or NaCl solution. Thus, the bicarbonate solution gave the lightest meat as compared with other washed samples. The Hunter **b** values, however, remained the same for all the different washed samples. The principal pigments responsible for the colour of dark meat are Mb and Hb. These pigments have also been shown to be extractable by water or $NaHCO_3$ solution (Saffle, 1973; Dawson *et al.*, 1988).

The Hunter **L** values for cooked MDCM are presented in Table 4.5. The effect of washing was significant only on Hunter **L** values and indicated that washed MDCM retained its light colour in the cooked state. The Hunter **L** value in unwashed samples was increased by 3.7 and 5.7% in the samples after the first and second washing with water, respectively. Washing with NaCl or $NaHCO_3$ solution or water (at pH 5.2) was also effective in producing lighter cooked washed MDCM. The bicarbonate solution was, however, most effective in producing the lightest colour (i.e., highest Hunter **L** value) in the products.

The Hunter **L**, **a** and **b** values of the samples were correlated with the total content of hemoproteins in raw unwashed and washed MDCM according to the regression equations given in Table 4.6. While both **L** and **a** values correlated well with the total content of hemoproteins present in the samples, Hunter **b** values did not show a good correlation.

Table 4.5 Hunter **L** values of cooked MDCM[1].

Treatment	Hunter **L** values
Unwashed MDCM	54.3 ± 0.3^{f}
Washed	
1 x H_2O	56.3 ± 0.3^{ce}
2 x H_2O	57.4 ± 0.2^{cd}
Washed once with H_2O, then	
0.5% NaCl	60.6 ± 0.2^{b}
0.5% $NaHCO_3$	62.2 ± 0.2^{a}
H_2O at pH 5.2	57.0 ± 0.2^{c}

[1]Results are mean values of 4 replicates ± standard deviation. Values with the same superscript are not significantly ($p>0.05$) different from one another.

Table 4.6 Correlation of regression equations relating Hunter raw colour parameters with total hemoproteins (T) in raw MDCM.

Parameters of regression equation Y = m + n * x				Correlation coefficient (r)
L	64.12	-3.39	T	-0.979
a	9.91	+2.91	T	+0.969
b	13.89	-0.11	T	+0.301

4.3 Amino acid composition, connective tissue content and protein efficiency ratio (PER)

The content of amino acids in the unwashed and washed MDCM is presented in Table 4.7. In general, there was a decrease in the content of some amino acids, however, the content of nonesssential amino acids alanine, arginine, aspartic acid, cysteine, hydroxyproline, proline and tyrosine was increased in some of the washed meat samples. This increase might be, in part, due to a relative increase in the content of connective tissues as indicated by the increased collagen content in all washed samples as compared with their unwashed counterparts (Table 4.7), and hence the contents of amino acids which are present in high levels in connective tissues such as hydroxyproline, proline and arginine were increased substantially as a result of various washings. The connective tissue content in unwashed increased by 98% and 146% in the samples washed with water and bicarbonate solution, respectively (Table 4.8). Variations in the content of amino acids bring about changes in the protein efficiency ratio (PER) values which may be calculated by the amino acid scoring methods developed by Lee *et al.* (1978).

The calculated PER values of both unwashed and washed MDCM are summarized in Table 4.8. A minor decrease in the PER values of all washed samples by 5.0 to 8.4%, based on equation 1, and 3.4 to 6.5%, based on equation 2, was evident. An increase in the content of nonessential amino acids in the resultant washed samples and enhanced content of connective tissues might be responsible for this observation. High levels of

Table 4.7 Amino acid composition of unwashed and washed MDCM (g/100 g crude protein)[1].

Amino acids	Unwashed MDCM	Washed MDCM			
		1xH_2O	1xH_2O, then 0.5% NaCl	1xH_2O, then 0.5% $NaHCO_3$	1xH_2O, then H_2O at pH 5.2
(Essential)					
Histidine	2.47±0.07^a	2.27±0.04^b	2.14±0.03^c	2.11±0.03^c	2.04±0.03^d
Isoleucine	5.38±0.16^a	5.28±0.13^a	5.37±0.03^a	5.37±0.02^a	5.31±0.04^a
Leucine	7.57±0.08^a	6.99±0.10^b	6.74±0.04^c	6.64±0.02^d	6.66±0.02^d
Lysine	8.38±0.08^a	8.11±0.02^b	7.96±0.02^c	7.82±0.01^c	7.91±0.01^c
Methionine	2.88±0.03^a	2.83±0.07^a	2.27±0.04^c	2.66±0.02^b	2.59±0.03^b
Phenyalanine	3.88±0.13^a	3.55±0.04^b	3.57±0.02^b	3.45±0.03^c	3.59±0.03^b
Threonine	4.55±0.06^a	4.35±0.01^b	4.21±0.03^c	4.14±0.06^c	4.25±0.02^c
Tryptophan	1.06±0.04^a	0.96±0.03^b	0.92±0.02^{bc}	0.83±0.01^c	0.91±0.01^b
Valine	5.52±0.03^a	5.31±0.01^b	5.25±0.02^c	5.03±0.03^d	5.16±0.03^e
(Nonessential)					
Alanine	5.88±0.09^c	6.12±0.01^b	6.15±0.01^b	6.34±0.02^a	5.67±0.01^d
Arginine	6.93±0.01^c	7.34±0.02^b	7.33±0.01^b	7.57±0.12^a	7.64±0.07^a
Aspartic acid[2]	8.57±0.03^c	8.88±0.05^b	8.97±0.03^b	9.09±0.04^a	8.14±0.04^d
Cysteine	1.36±0.03^c	1.57±0.06^b	1.43±0.05^b	1.50±0.04^b	1.72±0.02^a
Glutamic acid[3]	12.44±0.08^b	12.29±0.06^b	12.37±0.03^b	12.80±0.08^a	12.88±0.17^a
Glycine	6.90±0.04^a	6.91±0.03^a	6.92±0.04^a	6.73±0.02^b	6.65±0.01^c
Hydroxyproline	1.09±0.03^e	2.14±0.05^d	2.59±0.03^b	2.67±0.02^a	2.50±0.04^c
Proline	4.28±0.06^d	5.02±0.01^c	5.79±0.04^b	6.08±0.02^a	5.92±0.02^{ab}
Serine	3.82±0.06^a	3.47±0.03^b	3.51±0.01^b	3.30±0.02^c	3.48±0.02^b
Taurine	1.14±0.02^a	0.51±0.02^b	0.14±0.01^c	0.12±0.01^c	0.07±0.00^d
Tyrosine	3.36±0.02^b	3.49±0.03^a	3.28±0.02^c	3.08±0.01^d	3.26±0.02^c

[1]Results are mean values of 3 replicates ± standard deviation. Values in each row with the same superscript are not significantly ($p>0.05$) different from one another.

[2]Determined as aspartic acid + asparagine. [3] Determined as glutamic acid + glutamine.

Table 4.8 Predicted protein efficiency ratio (PER) values and connective tissue contents of unwashed and washed MDCM.

Treatment	PER Value[1]		Connective tissue content (g/100 g protein)[2]
	Equation 1	Equation 2	
Unwashed	2.98	2.92	8.75
Washed 1 x H_2O	2.83	2.82	17.18
Washed once with H_2O, then 0.5% NaCl	2.75	2.74	20.80
0.5% $NaHCO_3$	2.73	2.73	21.44
H_2O at pH 5.2	2.76	2.76	20.08

[1]Equation 1 : PER value = 0.08084 ΣAA_7 - 0.1094, where ΣAA_7 = Isoleucine + Leucine + Lysine + Methionine + Phenylalanine + Threonine + Valine.
Equation 2 : PER value = 0.06320 ΣAA_{10} - 0.1539, where ΣAA_{10} = ΣAA_7 + Arginine + Histidine + Tryptophan.

[2]Calculated as 8.03 x Hydroxyproline (Karatzas and Zarkadas, 1988).

collagen have been reported to be responsible for a lower content of total essential amino acids in washed samples (Lee *et al.*, 1978). However, both unwashed and washed MDCM showed a well-balanced amino acid composition in the products. The calculated PER values can only be used to compare the protein quality of washed meats with respect to their unwashed counterparts. Based on this, it is evident that the protein quality of MDCM will not be compromised due to aqueous washings.

4.4 Free amino acids and nucleotides/nucleosides

The content of free amino acids of both the unwashed and washed meats is shown in Table 4.9. Aqueous washings lowered the amount of all free amino acids in MDCM. Most free amino acids decreased by over 50.5% in the samples washed once with H_2O, and a further decrease was observed when meats were washed with 0.5% aqueous $NaHCO_3$. The free amino acids are water-soluble and hence are easily extracted into the aqueous media. The sulphur-containing amino acids, cysteine/cystine are important flavour precursors which have been implicated as precursors for volatile sulphur-containing heterocyclics with desirable flavour effects in meats (Shahidi, 1989). The content of cysteine decreased by 63.1 and 79.1% after one washing with H_2O or 0.5% $NaHCO_3$, respectively. A corresponding decrease of 51.3 and 80.7% was observed for the cystine content.

Table 4.9 The content of free amino acids of unwashed and washed MDCM (mg/100 g)[1]

Amino acid	Unwashed MDCM	Washed MDCM	
		1xH_2O	1xH_2O, then 0.5% $NaHCO_3$
Alanine	26.18±0.11	7.44±0.05	2.33±0.05
Anserine	153±1	49.71±0.08	15.21±0.10
Arginine	18.43±0.09	6.76±0.05	2.71±0.04
Aspartic acid[2]	24.81±0.12	7.15±0.04	2.67±0.03
Carnosine	15.30±0.09	5.26±0.04	2.02±0.02
Cysteine	2.25±0.02	0.83±0.06	0.47±0.01
Cystine	3.16±0.03	1.54±0.02	0.61±0.01
Glutamic acid[3]	67.14±0.24	17.26±0.07	5.45±0.03
Glycine	21.58±0.16	6.18±0.04	1.98±0.02
Histidine	8.72±0.04	2.55±0.02	0.95±0.02
Hydroxyproline	3.82±0.03	1.06±0.01	0.26±0.01
Isoleucine	9.44±0.03	2.67±0.02	0.97±0.02
Leucine	17.13±0.14	4.96±0.03	1.77±0.03
Lysine	27.65±0.16	8.42±0.05	3.37±0.03
Methionine	6.29±0.07	2.20±0.02	0.96±0.01
Phenylalanine	8.71±0.06	2.67±0.02	0.85±0.01
Serine	30.12±0.16	8.90±0.05	3.05±0.03
Taurine	116±1	38.36±0.08	12.53±0.11
Threonine	21.58±0.15	5.82±0.03	1.89±0.03
Tryptophan	2.31±0.02	0.83±0.01	0.29±0.01
Tyrosine	10.54±0.06	3.20±0.03	1.19±0.03
Valine	17.14±0.10	4.49±0.04	1.49±0.04
Total	611	188	63.0

[1]Results are mean values of 3 replicates ± standard deviation. Values in each row are significantly ($p>0.05$) different from one another.

[2] Determined from aspartic acid + asparagine.

[3] Determined from glutamic acid + glutamine.

The content of some nucleotides and nucleosides is given in Table 4.10. Washing brought about a substantial decrease in the concentration of inosine 5′-monophosphate (IMP), adenosine monophosphate (AMP), xanthine (X) and hypoxanthine (HX). The content of IMP was decreased from an initial level of 201 µg/g, by a factor of 9.0 after one washing with H_2O. Washing with $NaHCO_3$ solution further lowered the content of IMP by a factor of 14.3 in the resultant meat. The content of X and HX was also lowered by the two washings. ATP was not detected in the samples due to its breakdown prior to analysis. However, ADP was not significantly ($p>0.05$) lowered due to washing, while AMP was decreased by 57 and 91% due to washing with water and $NaHCO_3$ solution, respectively.

Free amino acids and nucleotides play an important role in the flavour development of meat products during heat processing. These compounds which are water-soluble are among the non-volatile precursors of meat flavour and are capable of interacting with reducing sugars and vitamins, or their breakdown products, thus leading to the formation of a large number of important volatile compounds which are essential for flavour development in cooked meats (Shahidi, 1989). Therefore, as expected, washed MDCM would have little flavour and is relatively bland in taste as compared with unwashed samples.

Table 4.10 Some nucleotide/nucleosides contents (µg/g) of unwashed and washed MDCM[1].

Treatment	ADP	AMP	IMP	X	HX
Unwashed MDCM	142±19[a]	7.4±1.0[a]	201±4[a]	24.0±0.3[a]	135±4[a]
Washed 1 x H_2O	132±13[a]	3.2±0.2[b]	20.3±0.3[b]	7.1±0.2[b]	48.1±1.9[b]
Washed once with water and then 0.5% $NaHCO_3$	123±15[a]	0.7±0.0[c]	13.1±0.3[c]	-	13.7±0.6[c]

[1]Results are mean values of 3 replicates ± standard deviation. Values each column with the same superscript are not significantly ($p>0.05$) different from one another. ATP was not detected in the samples.

4.5 Minerals

Table 4.11 summarizes the selected mineral content of unwashed and washed meats. Washing with $NaHCO_3$ had a pronounced effect in removing most minerals. The content of phosphorous, zinc, magnesium, iron and potassium was decreased by 56, 64, 67, 78 and 92%, respectively, in samples washed with 0.5% $NaHCO_3$ solution. Adu *et al.* (1983) have also reported a lower mineral content in surimi produced from minced fish flesh as compared with unwashed meat, since almost all minerals are water-soluble. The calcium and sodium content in the samples was, however, increased. This increase in calcium could be due to the concentration of residual bone particles in the washed meats, while washing with $NaHCO_3$ is responsible for the enhanced sodium content of the washed samples. Removal of iron from the muscle tissue may extend the shelf-life of the washed products. However, oxidation of ferrous to ferric compounds may also influence the rate of lipid oxidation in the resultant products.

4.6 Nucleic acids

The content of nucleic acids in both unwashed and washed MDCM is presented in Figure 4.1. The content of RNA of all samples was higher than that of DNA. The first washing with water (pH 6.9) decreased the content of DNA, RNA and the total nucleic acids in the samples by 32.2, 17.1 and 21.8%, respectively. $NaHCO_3$ solution was

Table 4.11 Selected minerals content of MDCM (mg/100 g)[1].

Minerals	Unwashed MDCM	Washed MDCM[2]
Calcium	78.4±3.1	84.2±4.1
Copper	0.1±0.0	< 0.1
Iron	1.8±0.2	0.4±0.0
Magnesium	17.7±0.5	5.8±0.1
Manganese	0.1±0.0	< 0.1
Phosphorus	195±4	86.4±3.7
Potassium	229±7	18.5±0.3
Sodium	86.8±4.2	123±3
Zinc	2.2±0.2	0.8±0.1
Total	611	319

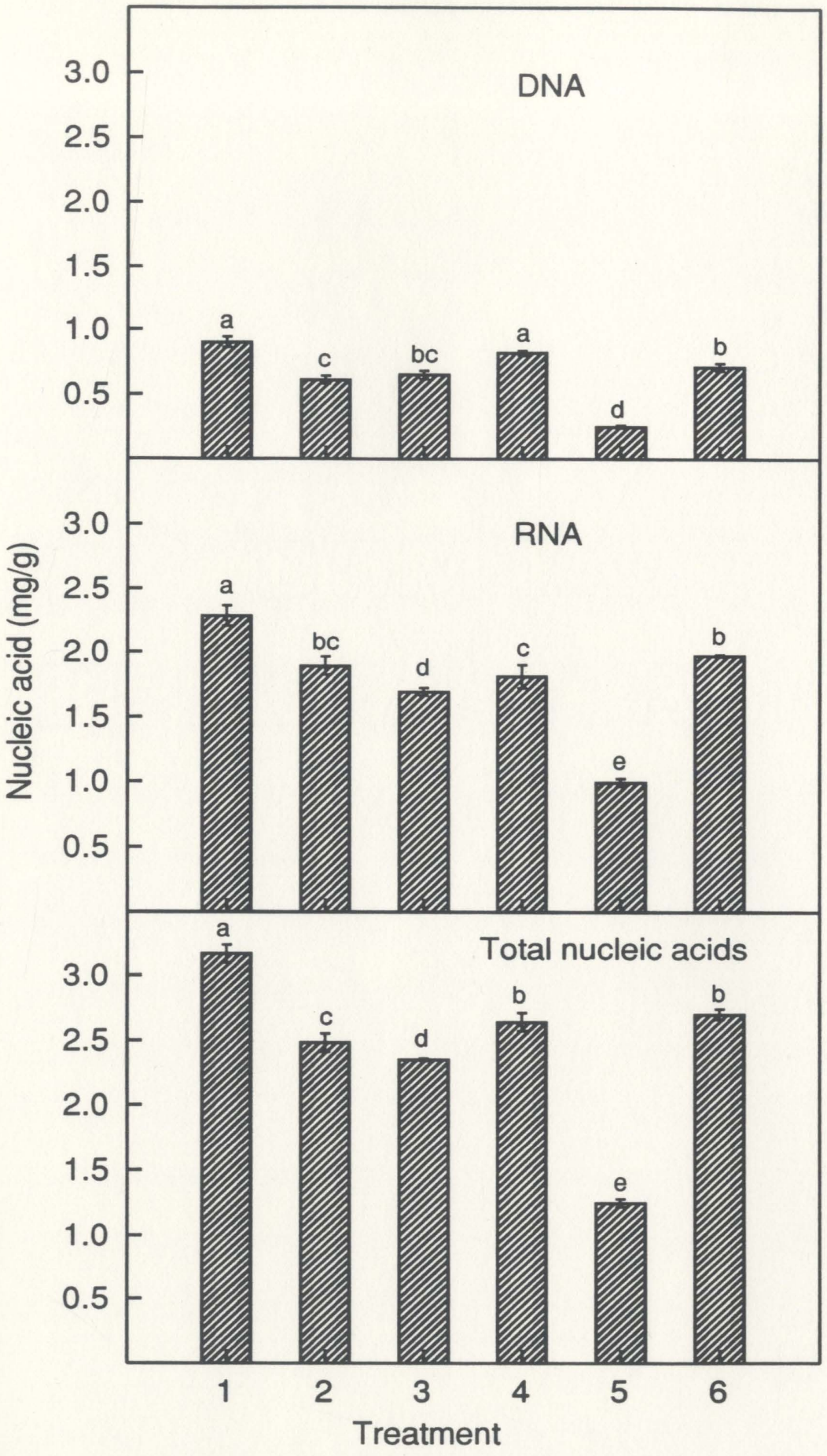
[1] Results are mean values of 3 replicates ± standard deviation.

[2] Samples were washed once with water and then with 0.5% $NaHCO_3$ solution.

most effective in extracting the nucleic acids from the samples by 60.6% from 3.17 to 1.25 mg/g. A relatively higher nucleic acid content in meat washed with water at pH 5.2 as compared with the second washing with water (pH 7.1) may be due to coprecipitation of nucleic acids with myofibrillar proteins at a pH close to their isoelectric point. Coprecipitation of chicken embryo RNA with myosin in low ionic salt solution has been reported by Heywood *et al.* (1968).

The content of nucleic acids in unwashed MDCM is slightly higher than a value of 2.75 mg/g reported by Savaiano *et al.* (1983) for mechanically deboned veal and values of 2.91 to 3.36 mg/g reported by Synowiecki and Shahidi (1992) for mechanically deboned seal meat. However, a value of 8.33 mg/g for mechanically deboned beef reported by Arusa *et al.* (1981) is much higher than those obtained in this study. A 2 g daily consumption of nucleic acids from foods is considered as the upper safe limit. Thus, 631 g of MDCM may be used without concern about their health-related drawbacks. Furthermore, different washings decreased the content of nucleic acids in the washed samples. The $NaHCO_3$ washing which was found to be most effective in decreasing hemoprotein and lipid contents, thereby producing a lighter meat, was also the most effective in decreasing the content of their nucleic acids.

Figure 4.1 Nucleic acids content of unwashed and washed MDCM.
1, Unwashed MDCM; Washed: 2, 1 x H_2O; 3, 2 x H_2O; 4, 1 x H_2O, then 0.5% NaCl; 5, 1 x H_2O, then 0.5% $NaHCO_3$; 6, 1 x H_2O, then H_2O (pH 5.2). Values with the same letter are not significantly ($p>0.05$) different from one another.

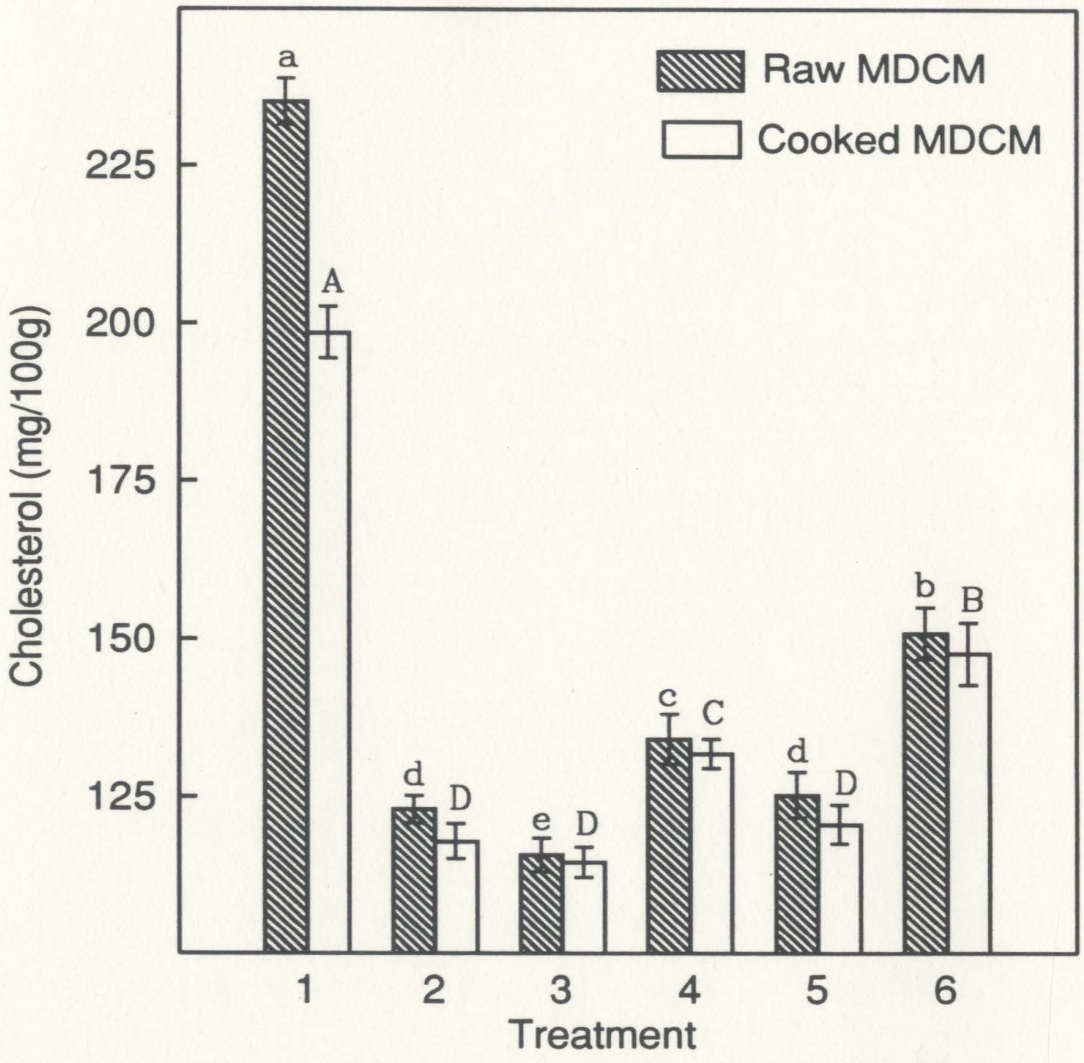

DNA
RNA
Total nucleic acids
Nucleic acid (mg/g)
Treatment
3.0
2.5
2.0
1.5
1.0
0.5
a
c
bc
a
d
b
a
bc
d
c
e
b
a
c
d
b
e
b
1
2
3
4
5
6

4.7 Cholesterol

The content of cholesterol in various samples after washing with different solutions is shown in Figure 4.2. The cholesterol content in raw meat was generally higher than that in cooked samples, but these differences were not significant except for the unwashed samples.

Since cooking was done in sealed pouches no loss of moisture and fat was possible, hence no change in the cholesterol content of samples was expected. Rhee *et al.* (1982a) reported an increase of 35-65% in the cholesterol content of cooked beef longissimus muscle steaks as compared to raw and attributed this apparent increase to the loss of moisture during the cooking process. Washing with different media was effective in reducing the fat content of the washed meat, and this may explain the lower cholesterol content in the washed samples. Both intermuscular and subcutaneous fat have been reported to contribute to the cholesterol content of beef steaks (Rhee *et al.*, 1982b). The efficiency of cholesterol removal in all washed meats was similar ($p>0.05$), except for the sample washed with H_2O at pH 5.2. The second washing with H_2O at pH 7.1 was most effective and decreased cholesterol content from 235 mg/100 g to 116 mg/100 g in the raw samples and from 198 to 114 mg/100 g in the cooked meats. Use of washed MDCM in emulsion-type products would result in reduced (40.8 to 57.8%) fat and cholesterol intake by consumers and affords a light-coloured product resembling breast meat.

Figure 4.2 Cholesterol content of raw and cooked MDCM as affected by washing. 1, Unwashed MDCM; Washed: 2, 1 x H_2O; 3, 2 x H_2O; 4, 1 x H_2O, then 0.5% NaCl; 5, 1 x H_2O, then 0.5% $NaHCO_3$; 6, 1 x H_2O, then H_2O (pH 5.2). Values with the same letter (lower case = raw, upper case = cooked) are not significantly ($p>0.05$) different from one another.


Raw MDCM
Cooked MDCM
Cholesterol (mg/100g)
225
200
175
150
125
a
A
d
D
e
D
c
C
d
D
b
B
1
2
3
4
5
6
Treatment

4.8 Sulphydryl groups and disulphide bonds

The results for the content of sulphydryl groups and disulphide bonds in unwashed and washed MDCM is presented in Table 4.12. Washing resulted in a decrease in the content of free sulphydryl groups of the meat as compared with the unwashed samples. A single washing with water resulted in a 14.1% decrease in content of sulphydryl groups and a second washing with water, 0.5% NaCl or 0.5% $NaHCO_3$ solution brought about a 19.2, 17.5 and 31.1% decrease, respectively. However, there was a proportionate increase in the disulphide bonds except for meat washed with H_2O at pH 5.2. The latter increase may be explained by the difference in the content of free sulphydryl groups and disulphide bonds between proteins, removed during washings from the samples and those present in the resultant meat. The second washing with water removed 28.1% of the proteins mostly sarcoplasmic, thus concentrating the myofibrillar and connective tissue proteins.

The effect of heating at various temperatures (20-99 °C) for 40 min on sulphydryl groups and disulphide bonds in the samples is given in Table 4.13. There was a progressive decrease in the content of free sulphydryl groups as temperature increased. Increasing the temperature from 20 to 99 °C increased the disulphide bonds content by a factor of 3.3, while the sulphydryl groups decreased by 60.3%. Oxidation of sulphydryl groups to disulphide bonds may be a major cause for these changes. Liu *et al.* (1982) reported a decrease of about 50% in total sulphydryl groups of croaker actomyosin at

Table 4.12 Sulphydryl groups and disulphide bonds content in MDCM proteins[1].

Treatment	Sulphydryl groups (μmol/g protein)		Disulphide bonds (μmol/g protein)[2]
	Initial sample	After reduction of disulphide bonds	
Unwashed MDCM	57.95±0.81[a]	76.23±0.51[a]	8.86±0.44[d]
Washed			
1 x H_2O	49.79±0.59[b]	68.75±0.41[c]	9.77±0.51[c]
2 x H_2O	46.85±0.26[c]	69.50±0.35[c]	10.98±0.63[bc]
Washed once with H_2O and then			
0.5% NaCl	47.83±0.25[c]	70.95±0.32[b]	11.21±0.71[b]
0.5% $NaHCO_3$	39.93±0.17[d]	67.19±0.34[d]	13.22±0.92[a]
H_2O at pH 5.2	49.14±0.73[b]	66.77±0.36[d]	8.54±0.61[d]

[1]Results are mean values of 6 replicates ± standard deviation. Value in each column with the same superscript are not significantly ($p>0.05$) different from one another.

[2]Disulphide bonds were calculated as [Total reduced SH - Free SH x 0.97]/2 (Synowiecki and Sikorski, 1988)

Table 4.13 Effect of heat processing (40 min) at various temperatures on sulphydryl groups and disulphide bonds in MDCM proteins[1].

Temperature, °C	Sulphydyl groups, (µmol/g protein)	Disulphide bonds, (µmol/g protein)
20	60.32 ± 1.77	7.71 ± 1.12
40	53.96 ± 1.59	10.79 ± 0.77
50	45.96 ± 1.28	14.85 ± 0.51
60	40.90 ± 0.55	17.40 ± 0.39
75	35.95 ± 0.78	19.63 ± 0.42
99	23.96 ± 0.57	25.35 ± 0.28

[1]Results are mean values of 6 replicates ± standard deviation. Values in each column are significantly different ($p<0.05$) from one another. The total SH content in MDCM after reduction was 76.23±0.51 µmoles/g protein.

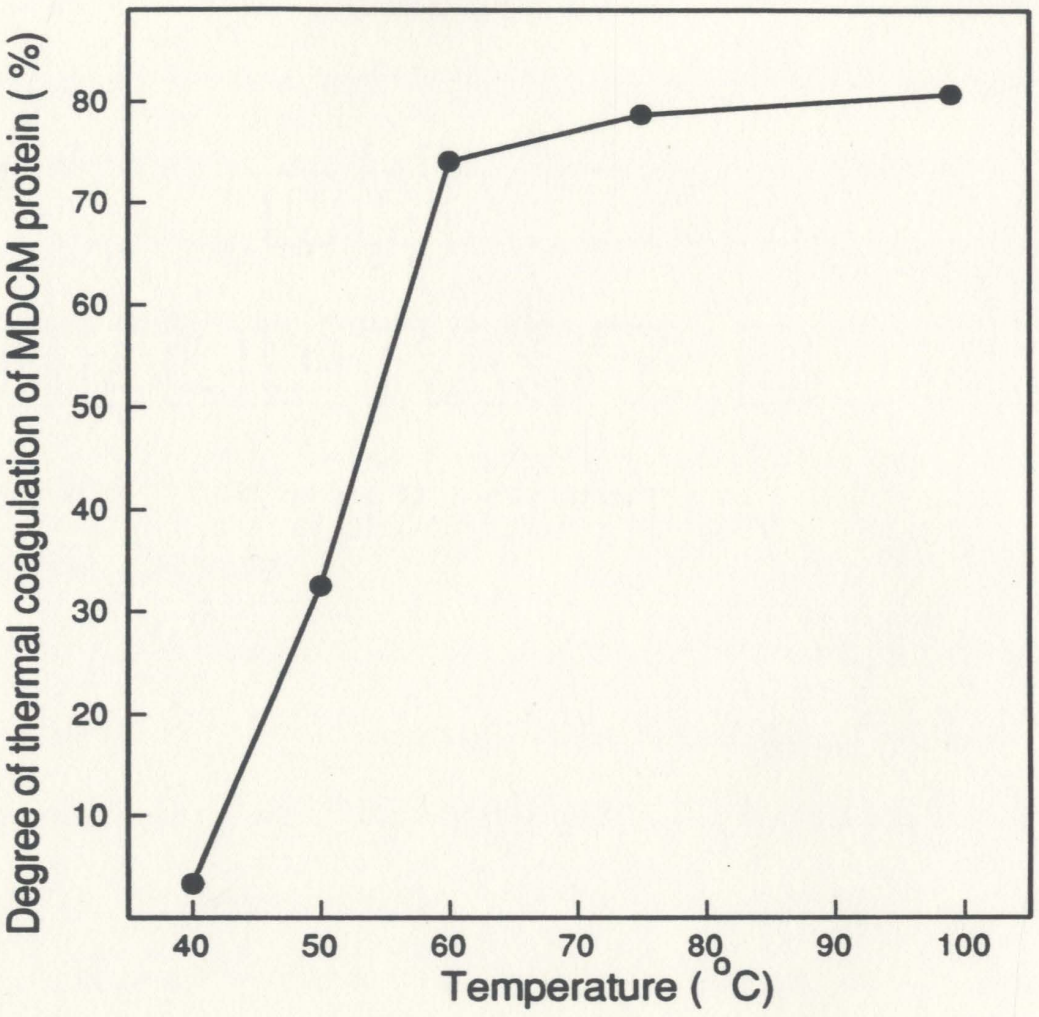
temperatures above 35 °C. The observed increase in disulphide bonds of MDCM proteins would most likely play an important role in gel formation properties of MDCM in processed meat products.

The extent of thermal denaturation of proteins during heating expressed as changes in their solubility in 5% NaCl solution was also monitored (Figure 4.3). A large decrease in the solubility of MDCM proteins and a corresponding increase in their degree of thermal coagulation was observed between 40 and 60 °C. However, between 60 to 99 °C there was only a slight change in this parameter. Similar results were reported by Liu *et al.* (1982) when croaker actomyosin solutions were heated for 30 min with an observed sharp rise in protein coagulation between 35 and 60 °C. Although oxidation of sulphydryl groups may have contributed to coagulation of MDCM protein, hydrophobic interactions may have also contributed to protein aggregation and coagulation (Liu *et al.*, 1982).

The influence of disulphide bond formation was also evidenced by comparison of the protein solubility in sodium dodecyl sulphate (SDS) with or without the addition of 2-mercaptoethanol, which reduces the disulphide bonds. Solubility of MDCM proteins expressed as a % of their amount in the meat (14.3%), after heating at 80 °C in SDS with or without 2-mercaptoethanol were 81.2 ± 0.6% and 88.3 ± 0.3%, respectively (Figure 4.4).

Formation of disulphide bonds during heating at different temperatures (X) was found to correlate well with the degree of thermal coagulation of MDCM proteins (Y),

Figure 4.3 Degree of thermal coagulation of proteins extracted from MDCM by 5% NaCl in 0.003M $NaHCO_3$ solution.


Degree of thermal coagulation of MDCM protein (%)
10
20
30
40
50
60
70
80
40
50
60
70
80
90
100
Temperature (°C)

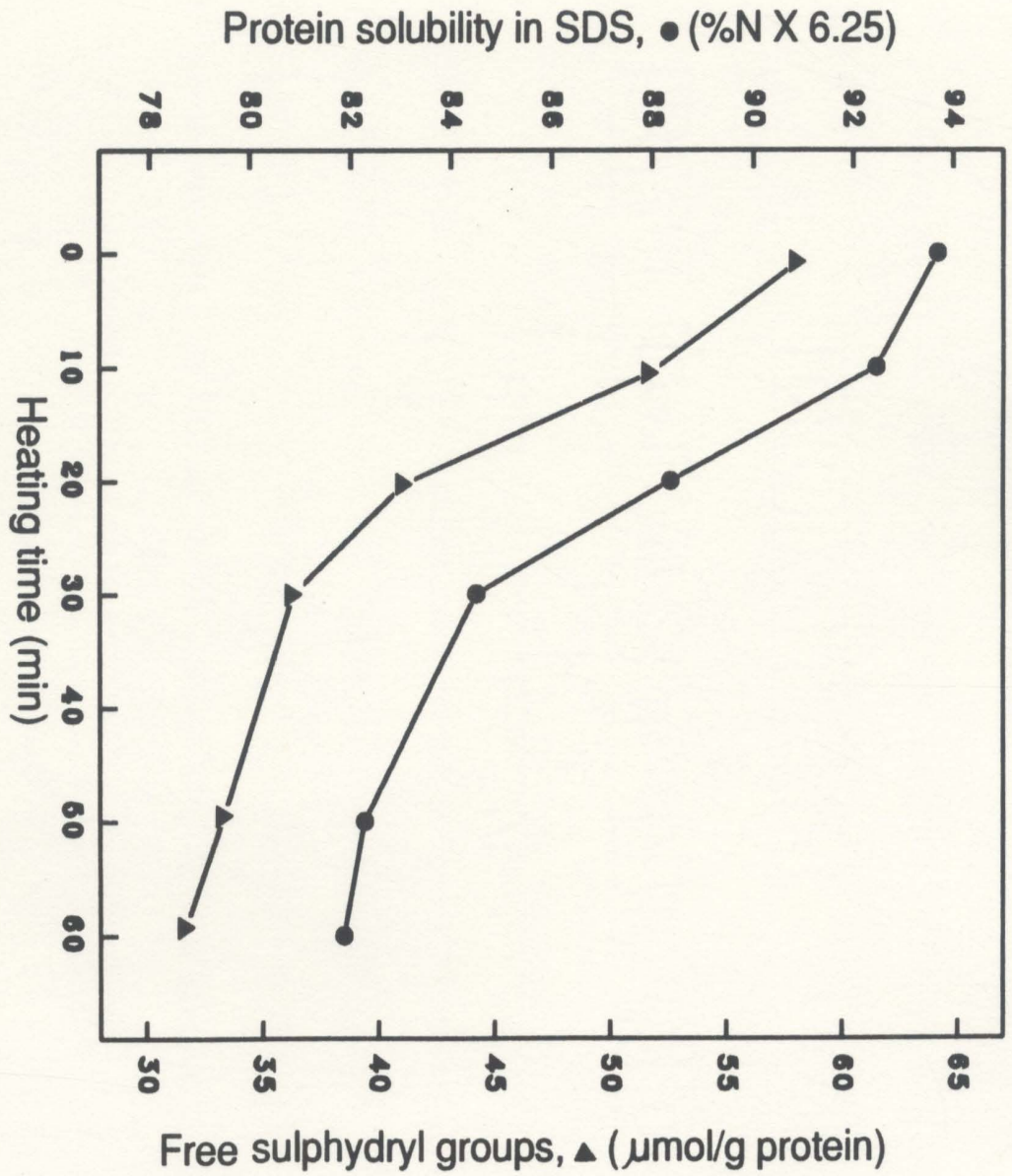
with a correlation coefficient of r = 0.917 for the regression equation Y = 5.383X. The content of sulphydryl groups also depended on the heating time (Figure 4.4). The decrease in the content of SH groups (X) in MDCM heated at 80 °C for up to 60 min also correlated well with the decrease in solubility of proteins in 0.035M SDS (Y), (r=0.980) for the regression equation Y = 67.31 + 0.472X. The decrease in free SH groups paralleled disulphide bond formation in the samples. There was no significant ($p>0.05$) difference in the content of SH groups found in raw (76.23 ± 0.51 µmol/g) and cooked (78.87 ± 0.63 µmol/g) proteins after reduction of disulphide bonds. Thus, one may conclude that no H_2S was produced in this process.

4.9 Functional properties

4.9.1 Percent cook yield

The % cook yield of unwashed and washed MDCM is shown in Table 4.14. Meat samples washed with $NaHCO_3$ solution gave the highest cook yield. At pH values above the isoelectric point of myofibrillar proteins (5.2-5.5), less attraction occurs between the myofibrillar filaments resulting in large interstitial space and hence entrapment of water molecules in the myofibrillar network and enhanced hydration of samples washed with a bicarbonate solution. About 80% of the water held in meat is ascribed to the water that is entrapped in the lattice spacings between the protein filaments of the myofibrils (Hamm, 1985), hence the pH condition of meat will have a

Figure 4.4 Effect of heat processing time of MDCM at 80 °C on protein solubility in 0.035M SDS and free sulphydryl groups content.


Protein solubility in SDS, ● (%N X 6.25)
78
80
82
84
86
88
90
92
94
Heating time (min)
0
10
20
30
40
50
60
30
35
40
45
50
55
60
65
Free sulphydryl groups, ▲ (μmol/g protein)

major influence on water retention and cook yield of the meat. Similar results were obtained for MDPM washed with a bicarbonate or phosphate solution (Dawson *et al.*, 1988). The cook yield of MDCM decreased by 19.6 and 18.6% after a one or a two-stage aqueous washing, respectively. Percent cook yield of the sample washed with water at pH 5.2 decreased by 11.4% as compared with unwashed MDCM. Water binding of meat proteins is important in enhancing juiciness, which is a unique and desirable feature of good quality meat products. Meats washed with bicarbonate solutions are therefore suitable as an ingredient in meat products.

4.9.2 Emulsion stability and emulsifying capacity

The emulsion stability and emulsifying capacity of unwashed and washed MDCM is shown in Table 4.15. The emulsion stability data indicates that meats washed with H_2O and $NaHCO_3$ solution produced slightly less stable emulsions than those from unwashed meats. The % stable emulsion formed in unwashed MDCM was 70.2±0.6%, and was decreased by 11.4 and 8.8% following washing with H_2O or bicarbonate solution, respectively. The lower stability of emulsions formed from washed meats may be attributed to lower protein content in the washed MDCM. Emulsion stability of MDPM containing 15% protein was higher than that containing 9% protein (Baker and Darfler, 1975). Increased content of collagen in the washed meats may also be a contributing factor in lowering the emulsion stability values. Meat with a high protein content would

Table 4.14 Percent cook yield of unwashed and washed MDCM[1]

Treatment	Percent cook yield
Unwashed MDCM	67.80±1.25[b]
Washed	
1 x H_2O	54.49±0.26[d]
2 x H_2O	55.15±0.69[d]
Washed once with H_2O, then	
0.5% NaCl	57.89±0.16[c]
0.5% $NaHCO_3$	72.10±1.10[a]
H_2O at pH 5.2	60.05±1.15[c]

[1]Results are mean values of 4 replicates ± standard deviation. Values in each column with the same superscript are not significantly ($p>0.05$) different from one another.

likely contain high amounts of salt-soluble proteins which contribute substantially to emulsion formation in the samples (Ahn *et al.*, 1981). Therefore, hand deboned breast meat with a high protein content (21.9%) produced the most stable emulsion as compared with unwashed and washed meats. Elkhalifa *et al.* (1988) also reported that breast meat tissues more effectively stabilized emulsion than unwashed and washed turkey thigh meat.

Emulsifying capacity of unwashed and washed MDCM were not significantly ($p>0.05$) different from one another (Table 4.15). However, McCready and Cunningham (1971) have reported larger emulsifying capacity values for various poultry meats at pH 7.0 than those at pH 5.0 or at the normal pH of meat (5.9-6.5). Therefore, removal of sarcoplasmic proteins has no adverse effect on emulsifying capacity of washed meats (Elkhalifa *et al.*, 1988). The expected relative concentration of myofibrillar proteins in the washed meats may also be responsible for the observed numerical increase in emulsifying capacity of washed meats. Emulsifying capacity of hand deboned breast meat was greater than those of unwashed and washed MDCM.

4.10 Fatty acid composition

4.10.1 Fatty acid profile of total lipids

The fatty acid composition of the total lipids of MDCM is shown in Table 4.16. Washing brought about a slight increase in the content of saturated fatty acids and a slight decrease in polyunsaturated fatty acids. The major fatty acids in the samples were oleic

Table 4.15 Emulsion stability and emulsifying capacity of unwashed and washed chicken meat[1].

Treatment	Emulsion stability (%)	Emulsifying capacity (mL oil/2.5 g)
Unwashed MDCM	70.2±0.6[b]	155±7[b]
Washed 1 x H_2O	63.0±0.3[c]	171±7[b]
Washed once with H_2O, then 0.5% $NaHCO_3$	64±0.7[c]	160±5[b]
HDCM - Breast	72.8±0.7[a]	190±8[a]

[1] Results are mean values of 3 replicates ± standard deviation. Values in each column with the same superscript are not significantly ($p>0.05$) from one another.

($C_{18:1}$), palmitic ($C_{16:0}$), linoleic ($C_{18:2}$), stearic ($C_{18:0}$) and palmitoleic ($C_{16:1}$) which constituted 95.5 to 96.8% of the total fatty acids in unwashed and washed meats. The total crude lipid content of unwashed MDCM was 10.1% while that of meat washed with a $NaHCO_3$ solution was 4.9%. Neutral lipids constituted approximately 96.5% of the total lipids while phospholipids made up the remainder of lipids in the unwashed meat. There was an increase in phospholipids relative to the neutral lipids in washed MDCM. The content of phospholipids in MDCM washed with H_2O and $NaHCO_3$ averaged 12.9% and 15.1%, respectively.

4.10.2 Fatty acid profile of the neutral lipid fraction

The fatty acid profile of the neutral lipids was similar to that of the total lipids (Table 4.17). This is due to the high proportion of neutral lipids in the total crude lipids. Simon and Gandemer (1986) reported a similar trend for lipids of MDCM. Other workers have also found oleic and palmitic acids in high concentration in MDCM (Jantawat and Dawson, 1979; Moerck and Ball, 1974). Low levels of polyunsaturated fatty acids were found in the neutral lipid fraction. Moerck and Ball (1974) reported similar results for the non-polar fatty acids of chicken breast, thigh muscles, skin and MDCM. The effect of washing on the fatty acids of non-polar lipids fraction of MDCM was similar to that of total lipids (see Table 4.17). The neutral lipid fraction of meats makes a minimal contribution to its oxidation (Igene and Pearson, 1979). The fatty acid composition of the

Table 4.16 Fatty acid profile of total lipids from unwashed and washed MDCM[1].

Fatty acid[2]	Unwashed MDCM	Washed MDCM	
		$1xH_2O$	$1xH_2O$, then 0.5% $NaHCO_3$
14:0	0.96	1.69	0.78
14:1	0.20	0.15	0.15
16:0	23.45	23.66	27.09
16:1	6.17	6.43	5.64
18:0	7.33	7.81	7.39
18:1	46.02	45.86	45.58
18:2	12.55	12.19	11.14
18:3	1.86	0.53	0.53
20:0	0.12	0.14	0.18
20:2	0.34	0.61	0.54
20:3	0.16	0.19	0.12
20:4	0.84	0.74	0.86
20:5	trace	trace	trace
22:5	trace	trace	trace
22:6	trace	trace	trace
Saturates	31.86	33.30	35.44
Monounsaturates	52.39	52.44	51.37
Polyunsaturates	15.75	14.26	13.19

[1] Results are average values of 2 replicates

[2] Number of carbon atoms:number of double bonds.

Table 4.17 Fatty acid profile of neutral lipids from unwashed and washed MDCM[1].

Fatty acid[2]	Unwashed MDCM	Washed MDCM	
		1xH_2O	1xH_2O, then 0.5% $NaHCO_3$
14:0	0.93	1.61	0.72
14:1	0.18	0.16	0.14
16:0	23.37	26.01	26.56
16:1	6.15	6.08	5.81
18:0	7.27	6.47	7.17
18:1	46.17	45.60	46.36
18:2	12.57	11.88	11.19
18:3	1.87	0.56	0.59
20:0	0.14	0.12	0.23
20:2	0.37	0.69	0.63
20:3	0.18	0.19	0.16
20:4	0.78	0.63	0.44
Saturates	31.71	34.21	34.68
Monounsaturates	52.50	51.84	52.31
Polyunsaturates	15.77	13.95	13.01

[1] Results are average values of 2 replicates.

[2] Number of carbon atoms:number of double bonds.

neutral lipids contained more saturated and short-chain unsaturated fatty acids which are less susceptible to oxidative degradation, hence the contribution of neutral lipids to oxidative deterioration of chicken lipids is less important as compared to that of phospholipids.

4.10.3 Fatty acid profile of the phospholipid fraction

The fatty acid composition of the phospholipids fraction of MDCM lipids is given in Table 4.18. Results indicate that the fatty acid composition of this fraction is quite different from that of the total and neutral lipids. The predominant fatty acids of the phospholipids in the unwashed and washed MDCM were oleic ($C_{18:1}$), palmitic ($C_{16:0}$) and linoleic ($C_{18:2}$). The total saturated fatty acids slightly decreased due to washing with either H_2O or $NaHCO_3$ solution, while the polyunsaturated fatty acids increased. The presence of polyunsaturated fatty acids with 3 to 6 double bonds was conspicuous in the fatty acid profile of the phospholipid fraction. These long-chain (C_{18} to C_{22}) polyunsaturated fatty acids accounted for 9.51 to 11.01% of the total fatty acids of the phospholipids as compared with 1.23 to 1.33% in the neutral lipids fraction. Presence of large proportion of highly unsaturated fatty acids (HUFA), linoleic acid ($C_{18:2}$) and arachidonic acid ($C_{20:4}$) is a distinctive characteristic of chicken meat phospholipids (Lee and Dawson, 1973). The polyunsaturated fatty acids are highly prone to oxidation. It is often assumed that oxidative deterioration of tissue lipids is primarily due to oxidation of

Table 4.18 Fatty acid profile of phospholipids from unwashed and washed MDCM[1].

Fatty acid[2]	Unwashed MDCM	Washed MDCM	
		1xH_2O	1xH_2O, then 0.5% $NaHCO_3$
14:0	0.44	1.29	0.54
14:1	0.12	0.10	0.10
16:0	23.71	21.65	22.25
16:1	2.39	3.14	2.27
18:0	17.40	17.40	17.50
18:1	29.19	29.19	28.05
18:2	14.86	14.86	15.60
18:3	0.37	0.54	0.32
20:0	0.52	0.54	0.48
20:2	0.35	0.43	0.56
20:3	0.57	0.67	0.79
20:4	4.10	4.32	4.61
20:5	trace	trace	trace
22:0	0.48	0.51	0.55
22:4	1.41	1.67	1.55
22:5	0.92	1.07	1.31
22:6	1.73	2.01	2.19
24:0	trace	trace	trace
24:1	1.44	1.10	1.33
Saturates	42.55	41.27	41.32
Monounsaturates	33.14	33.15	31.75
Polyunsaturates	24.31	25.58	26.93

[1] Results are average values of 2 replicates.

[2] Number of carbon atoms:number of double bonds.

phospholipids. Lee and Dawson (1973) have shown that the phospholipids in chicken muscles and skin had the greatest effect on lipid deterioration based on their loss of fatty acids during cooking and frozen storage even when a much greater amount of neutral lipids were present in these tissues. The inefficient removal of polyunsaturated fatty acids during washing of MDCM may have minor effects in enhancing the oxidative and flavour stability of washed MDCM.

4.10.5 Individual phospholipids

The separation of individual phospholipids of MDCM on a TLC plate is represented in Figure 4.5. The phospholipid classes identified were phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SPH) and lysophosphatidylcholine (LPC). The solvent front (SF) contains phosphatidic acid, phosphatidylglycerol, cardiolipin, cholesterol and trace levels of triglycerides. The proportions of these phospholipids in the MDCM were in agreement with other reports. Of the compounds separated and identified, PC was very dominant followed by PE, SPH, PS and LPC (Table 4.19). Similar findings were reported by Pikul and Kummerow (1990).

The high proportions of PC and PE are characteristic of chicken muscle fat (Lee and Dawson, 1976). Jantawat and Dawson (1980) have reported that PC, PE, SPH and PS are the predominant components of muscle and bone phospholipids and comprised

Figure 4.5 Diagram of a thin-layer chromatogram showing separation of the classes of MDCM phospholipids.
SF, solvent front (phosphatidic acid, phosphatidylglycerol, cardiolipin, cholesterol and triglycerides); PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; SPH, sphingomyelin; LPC, lysophosphatidylcholine.

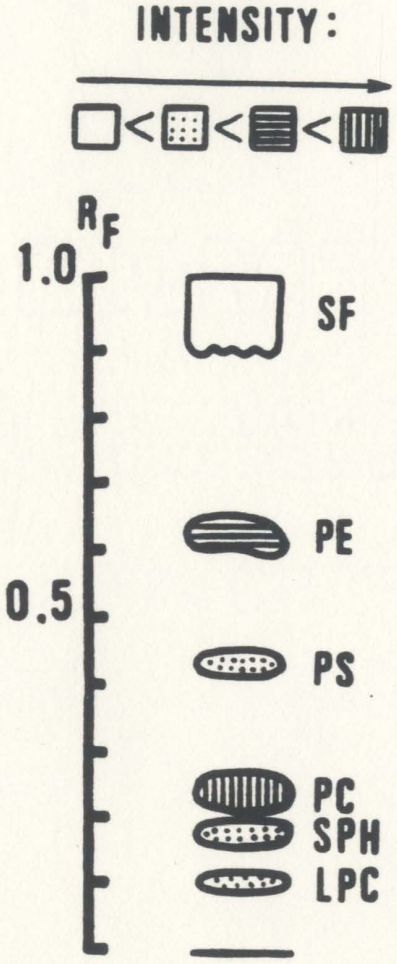

INTENSITY:
R_F
1.0
0.5
SF
PE
PS
PC
SPH
LPC

Table 4.19 Percentage phospholipid (PL) concentration relative to the total PL[1].

Phospholipid[2]	Unwashed MDCM	Washed MDCM	
		$1xH_2O$	$1xH_2O$, then 0.5% $NaHCO_3$
SF	5.1 ± 0.1^{fA}	5.2 ± 0.1^{eA}	5.0 ± 0.1^{eA}
PE	15.8 ± 0.2^{bA}	16.5 ± 0.3^{bA}	16.4 ± 0.3^{bA}
PS	8.1 ± 0.1^{dA}	8.5 ± 0.2^{dA}	8.6 ± 0.1^{dA}
PC	53.9 ± 0.4^{aA}	51.6 ± 0.5^{aB}	51.5 ± 0.6^{aB}
SPH	9.9 ± 0.2^{cA}	10.0 ± 0.2^{cA}	10.2 ± 0.2^{cA}
LPC	7.2 ± 0.2^{eA}	8.2 ± 0.1^{dB}	8.3 ± 0.1^{dB}

[1] Results are mean values of 3 replicates ± standard deviation. Values with the same superscript in each column$^{(a-f)}$ and row$^{(A-B)}$ are not significantly ($p>0.05$) different from one another.

[2] SF, solvent front (phosphatidic acid, phosphatidylglycerol, cardiolipin, cholesterol and triglycerides); PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; SPH, sphingomyelin; LPC, lysophosphatidylcholine.

approximately 90% of the total phospholipids. Washing was effective in lowering PC by 4.3 and 4.5% in meats when using H_2O and a bicarbonate solution, respectively. A corresponding increase in the content of PE, SPH and PS was observed.

In general, an increase in the content of phospholipid groups in the washed samples was observed. The extent of susceptibility of individual phospholipids to oxidation was reported by Pikul and Kummerow (1990) who noted that PC and PE produced 70-77% of the total amount of phospholipid malonaldehyde from the phospholipid fraction of chicken lipids during 24 h storage.

4.11 Oxidative stability

The 2-thiobarbituric acid (TBA) number, defined as mg of malonaldehyde equivalents per kg of sample, of raw MDCM and hand deboned chicken meat (HDCM) as affected by washing during a 2-wk storage period at 4 °C are shown in Table 4.20. Results generally indicate a wide variation in the TBA values of meats from different parts of birds and those deboned mechanically versus manually. The TBA values of MDCM samples were generally higher than values obtained for HDCM. Higher content of polyunsaturated fatty acids and hemoproteins in MDCM as compared with HDCM (Moerck and Ball, 1973; Pikul and Niewiarowicz, 1988) may be responsible for this observation. The TBA numbers of raw $NaHCO_3$ washed MDCM from necks and backs increased as compared with their unwashed counterparts by a factor of 1.8 as compared

Table 4.20 2-Thiobarbituric acid (TBA) values (mg malonaldehyde equivalents/kg sample) of raw samples stored at 4 °C over a 2 wk period[1].

Meat sample	Treatment[2]	Storage period (days)				
		0	3	7	10	14
HDCM (necks and backs)	1	0.11	0.29	0.20	0.21	0.18
	2	0.21	0.44	0.46	0.30	0.22
	3	0.57	0.57	0.44	0.44	0.49
HDCM (legs and backs)	1	0.18	0.18	0.15	0.17	0.16
	2	0.18	0.15	0.14	0.17	0.15
	3	0.56	0.58	0.48	0.63	0.73
HDCM (breast)	1	0.21	0.16	0.10	0.18	0.17
	2	0.53	0.80	0.97	1.03	0.97
	3	0.50	1.06	0.48	0.53	0.46
MDCM (necks and backs)	1	0.22	0.30	0.29	0.33	0.61
	2	0.20	0.21	2.67	2.73	2.29
	3	0.16	0.27	2.44	2.53	1.93
MDCM (legs and backs)	1	0.26	0.91	0.41	0.86	1.12
	2	0.32	0.26	0.43	0.40	0.34
	3	0.46	0.38	0.87	0.64	0.72
MDCM (breast and backs)	1	0.08	0.16	1.02	0.24	0.30
	2	0.23	0.36	3.58	3.06	2.11
	3	0.64	1.14	1.55	1.26	1.03

[1] Results are average values of 2 replicates.

[2] 1, Unwashed; Washed: 2, 1 x H_2O; 3, 1 x H_2O, then 0.5% $NaHCO_3$.

with a factor of 1.6 for the hand deboned chicken meat.

The washed meats also showed oxidative deterioration during the storage period. The TBA value of MDCM (necks and backs) washed with $NaHCO_3$ solution was increased by 1480% from an initial value of 0.16. The mixing involved during washing may have brought the lipids into contact with prooxidants such as hemoproteins in the meat (Dawson *et al.*, 1989). Preferential removal of neutral lipids during washing as compared to phospholipids (see Section 4.10.1) may have also contributed to higher TBA values in washed meats. The phospholipids, due to their high degree of unsaturation, are the components of meat lipids which contribute primarily to oxidative deterioration of meat lipids and the development of warmed-over flavour in cooked products (Pikul *et al.*, 1984; Igene and Pearson, 1979). The neutral lipids constitute the bulk of fats which are preferentially removed from the samples thus, concentrating the phospholipids relative to neutral lipids in the washed meats.

The TBA values of cooked unwashed and washed MDCM and HDCM are presented in Table 4.21. The TBA values of cooked meats were in general much higher than those of uncooked products. Thus, warmed-over flavour development in cooked poultry meat is faster than the rate of normal lipid oxidation in raw meats and lends further support to the findings of Gray and Pearson (1987). The TBA values of cooked MDCM indicates oxidative instability of the unwashed and washed meats. However, washing with H_2O and $NaHCO_3$ were effective in producing cooked products with a lower

Table 4.21 2-Thiobarbituric acid (TBA) values (mg malonaldehyde equivalents/kg sample) of cooked samples stored at 4 °C over a 2 wk period[1].

Meat sample	Treatment[2]	Storage period (days)				
		0	3	7	10	14
HDCM (legs and backs)	1	5.67	10.41	12.01	9.48	8.22
	2	0.86	8.44	8.59	7.48	6.91
	3	4.57	0.72	0.98	1.33	1.87
HDCM (breast)	1	6.58	12.1	13.5	10.4	11.2
	2	4.23	5.91	6.29	5.05	4.90
	3	1.00	0.89	0.86	0.72	0.81
MDCM (necks and backs)	1	1.60	4.44	6.17	5.26	6.39
	2	0.74	1.76	2.52	1.93	2.05
	3	0.52	1.32	1.80	1.29	2.01
MDCM (legs and backs)	1	1.59	6.69	8.79	6.71	7.00
	2	0.49	2.15	2.65	2.30	2.91
	3	0.56	2.17	2.96	2.39	3.25
MDCM (breast and backs)	1	0.98	4.27	5.72	5.03	6.09
	2	0.45	1.28	1.05	1.51	1.89
	3	0.46	0.66	1.08	0.91	1.38

[1] Results are average values of 2 replicates.

[2] 1, Unwashed; Washed: 2, 1 x H_2O; 3, 1 x H_2O, then 0.5% $NaHCO_3$.

rate of off-flavour development as indicated by their TBA values. The TBA numbers of cooked unwashed MDCM (necks and backs) increased by a factor of 3.0 from an initial value of 1.60 during a 2-wk storage at 4 °C. The TBA value of the meat washed with a $NaHCO_3$ solution was increased by a factor of 2.8 from an initial value of 0.52 over the same period. This general trend for lower TBA values of cooked washed meats may be due to lower amounts of hemoproteins and free iron in cooked washed meats. Hemoproteins are a known source of free iron, which are released during cooking which accelerate lipid oxidation in the meats (Igene *et al.*, 1979; Chen *et al.*, 1984). The sensory evaluation of the cooked meat was not carried out, however, TBA value of approximately one have been related to the threshold level of rancidity in pork (Watts, 1962). If one would relate this value to MDCM, most unwashed and washed samples have reached such a threshold level within 0-3 days of storage at 4 °C.

CONCLUSIONS

Washing of MDCM with aqueous solutions brought about definite changes in the compositional characteristics of the products. Differences in total protein in the unwashed and washed meats were considered to be due to the removal of sarcoplasmic proteins. However, the amino acid composition and predicted protein efficiency ratio of unwashed and washed meats were similar. Loss of hemoproteins and total lipids as a result of washing of MDCM with a 0.5% $NaHCO_3$ solution was maximal and produced a light-coloured and low-fat product which may potentially be used as a breast-meat substitute in product formulations. The cook yield of the resultant washed meats was improved, but the emulsifying capacity and the emulsion stability were not enhanced in the washed MDCM.

Washing of MDCM also removed a considerable proportion of nucleic acids and cholesterol in the samples. However, some desirable flavour precursors such as free amino acids and nucleotides were also extracted from MDCM. Despite the removal of hemoproteins in washed meats, the raw washed samples had lower oxidative stability as compared with their unwashed counterparts and this was attributed to the concentration of phospholipids in the washed meat samples. However, cooked washed meats had better oxidative stability than their raw washed counterparts.

Further study is needed on the functional properties of the washed MDCM especially with respect to viscosity, textural and gel formation properties which would

enable proper assessment of the products for possible incorporation of washed meat in a wide variety of fabricated products. Research on improving the yield during the washing process and enhancing oxidative stability of washed MDCM is also needed.

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APPENDIX

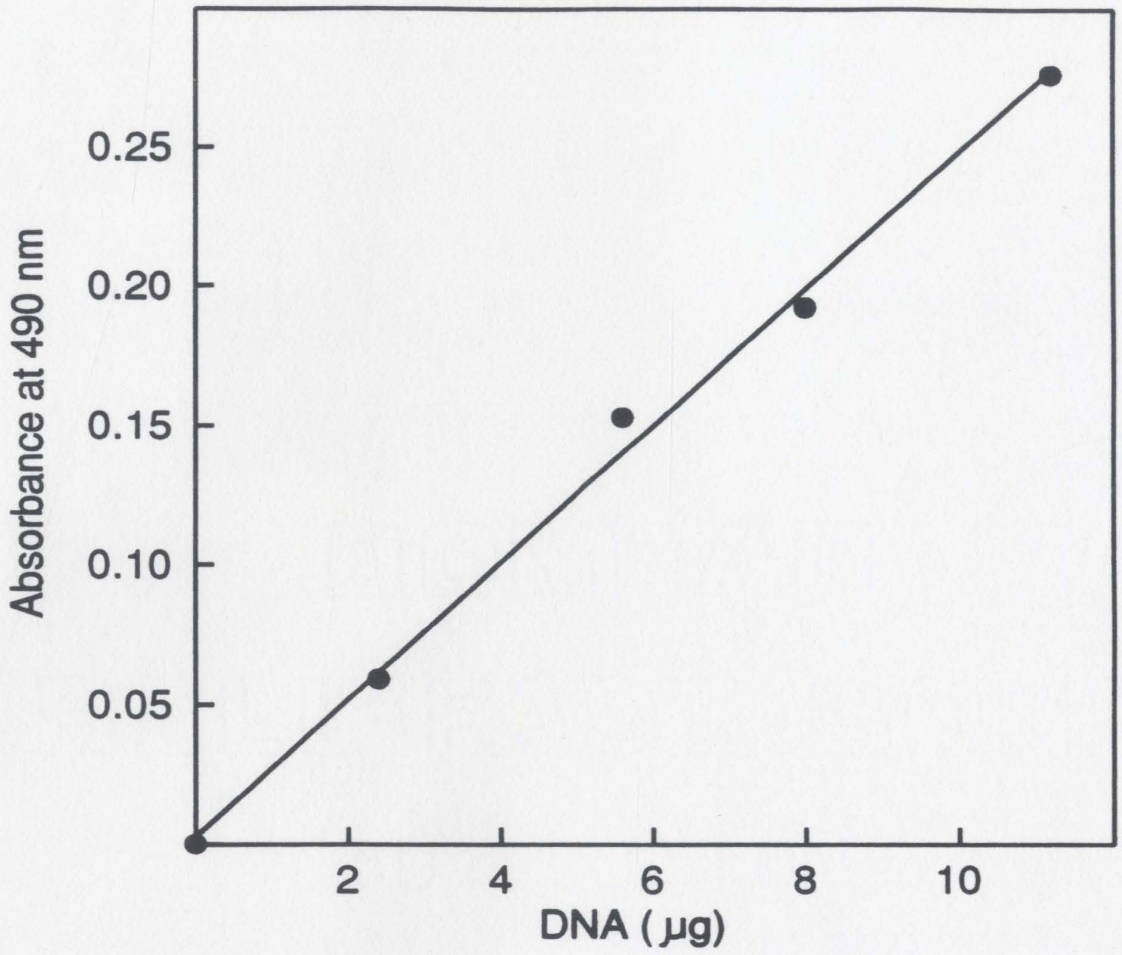

Figure A.1 Graph of standard line of deoxyribonucleic acid (DNA) concentration dependance on absorbance at 490 nm.

A.1 Deoxyribonucleic acid (DNA) estimation

Regression coefficient (r) = 0.998
Equation of the line was Y = aX + b where,
Y = absorbance at 490 nm (A_{490})
X = concentration of DNA in µg (C) in 1 mL extract
a = 0.024
b = 0.0
A_{490} = 0.024*C
Since 5 mL of the initial 100 mL of homogenate was used for analysis, therefore, the concentration of DNA (mg) in 50 mL total extract = $41.66A_{490}$
Since W grams of meat was used for the analysis, DNA content in sample
= $41.66A_{490}/W$ (mg/g sample).

A.2 Ribonucleic acid (RNA) estimation

1 mg RNA = 16.5 absorbance units at 260 nm

$A_{260} = 0.16*C$

Hence $C = 0.016A_{260}$

Since 5 mL of the initial 100 mL homogenate was used for analysis, therefore, the concentration of RNA (mg) in 100 mL extrtact = $60.6A_{260}$.

Since W grams of meat was used for the analysis, RNA content in sample $= 2*60.6A_{260}/W$ (mg/g sample).

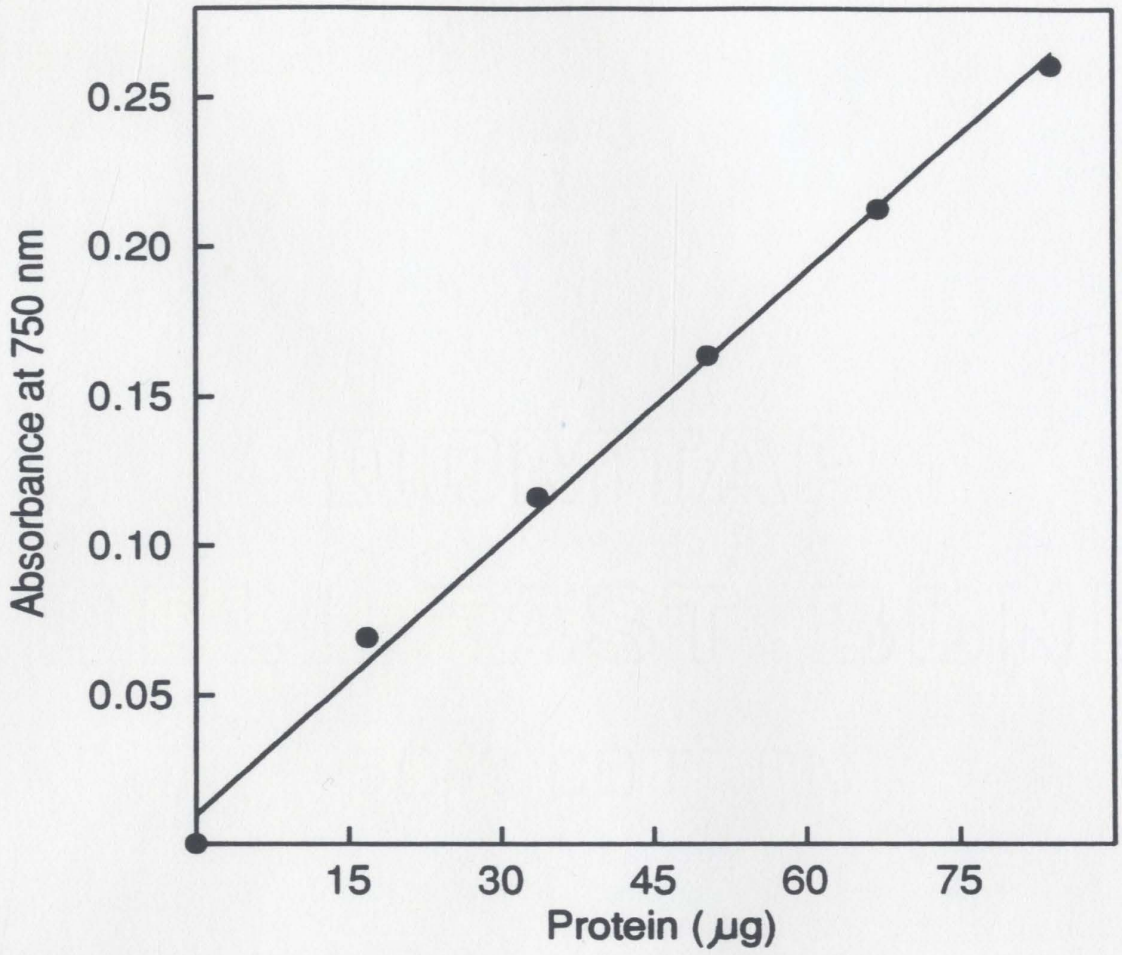

Figure A.3 Graph of standard line of protein concentration dependance on absorbance at 750 nm.

A.3 Protein estimation

Regression coefficient (r) = 0.995
Equation of the line was Y = aX+b where
Y = absorbance at 750 nm (A_{750})
X = concentration of protein in µg (C)
a = 0.003
b = 0.009
A_{750} = 0.003 + 0.009*C
Therefore, C = 83.33A_{750} (µg protein)

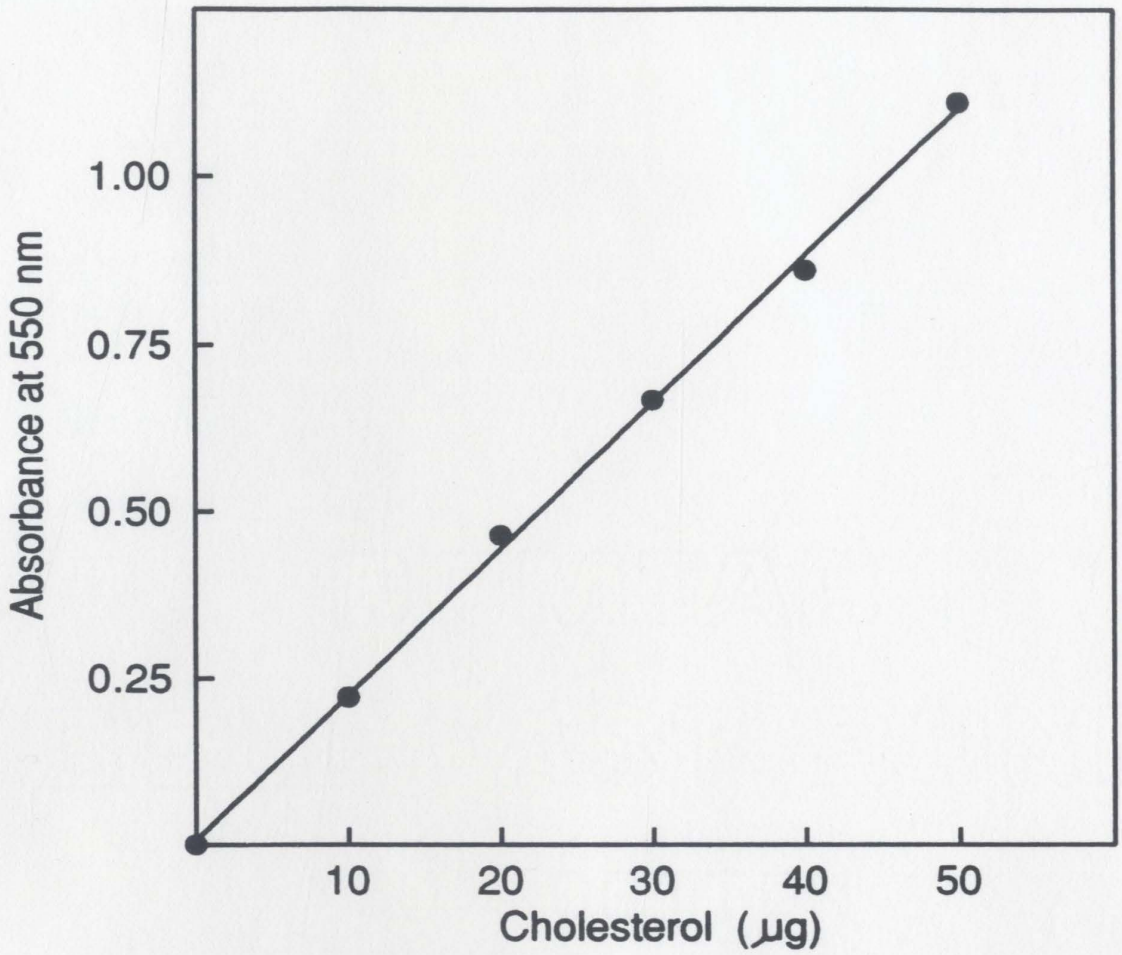

Figure A.4 Graph of standard line of cholesterol concentration dependance on absorbance at 550 nm.

A.4 Cholesterol estimation

Regression coefficient (r) = 0.992
Equation of the line was Y = aX+b where,
Y = absorbance at 550 nm (A_{550})
X = concentration of cholesterol in µg (C)
a = 0.022
b = 0.0
A_{550} = 0.022*C
Therefore, concentration of cholesterol (µg) in 0.2 mL $CHCl_3$ extract was,
$C = 45.45A_{550}$
Hence, cholesterol content (mg/100g) sample = $45.45A_{550}$*F
where F = Total volume of $CHCl_3$ extract x Volume of hexane /Volume of $CHCl_3$ used for analysis x 1000 x weight of sample (g).

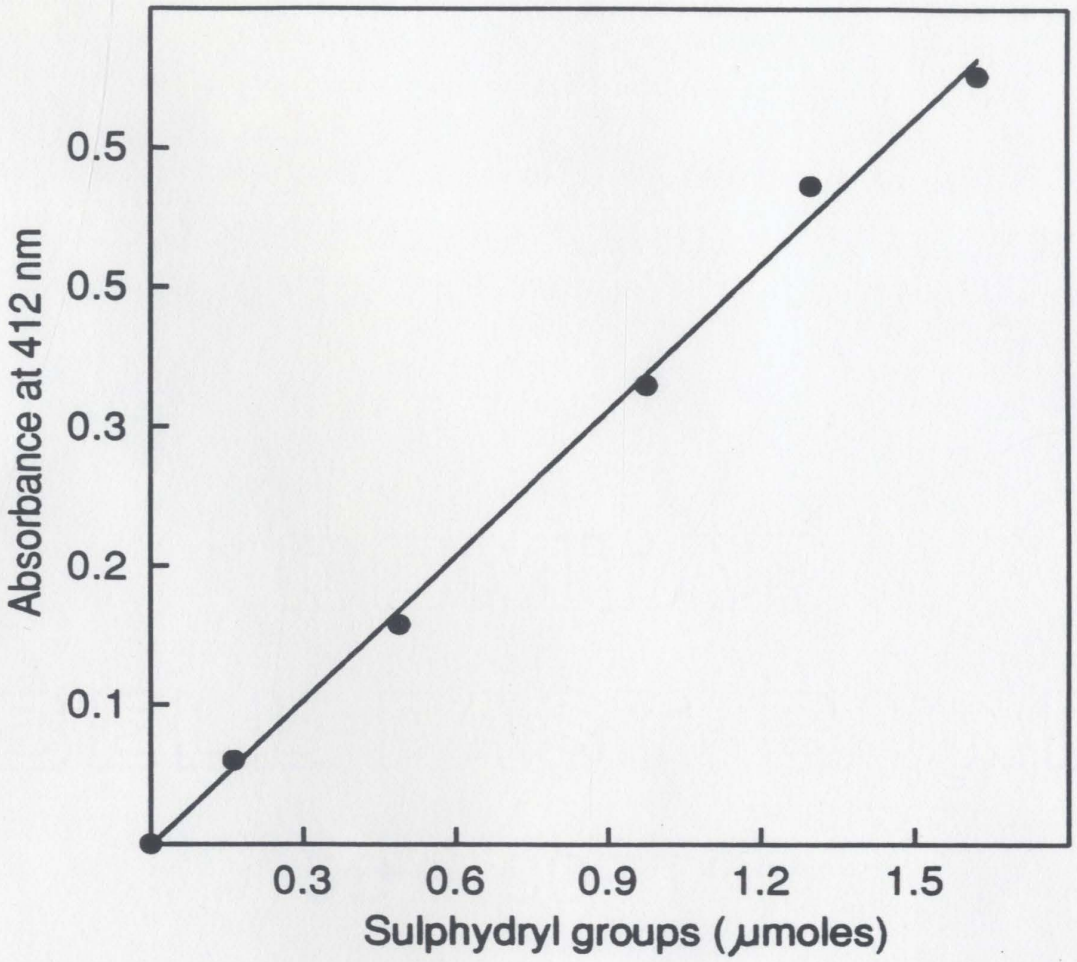

Figure A.5 Graph of standard line of sulphydryl groups concentration dependance on absorbance at 412 nm.

A.5 Sulphydryl groups estimation

Regression coefficient (r) = 0.998
Equation of the line was Y = aX+b where,

Y = absorbance at 412 nm (A_{412})
X = concentration of sulphydryl groups (SH) in µmoles (C)
a = 0.339
b = 0.0

$A_{412} = 0.339*C$
Therefore, $C = 2.95A_{412}$
Since M grams of meat was used for the analysis and protein content of the sample was Z, Sulphydryl group content in sample = $2.95A_{412}/MZ$ (µmoles SH/g protein).

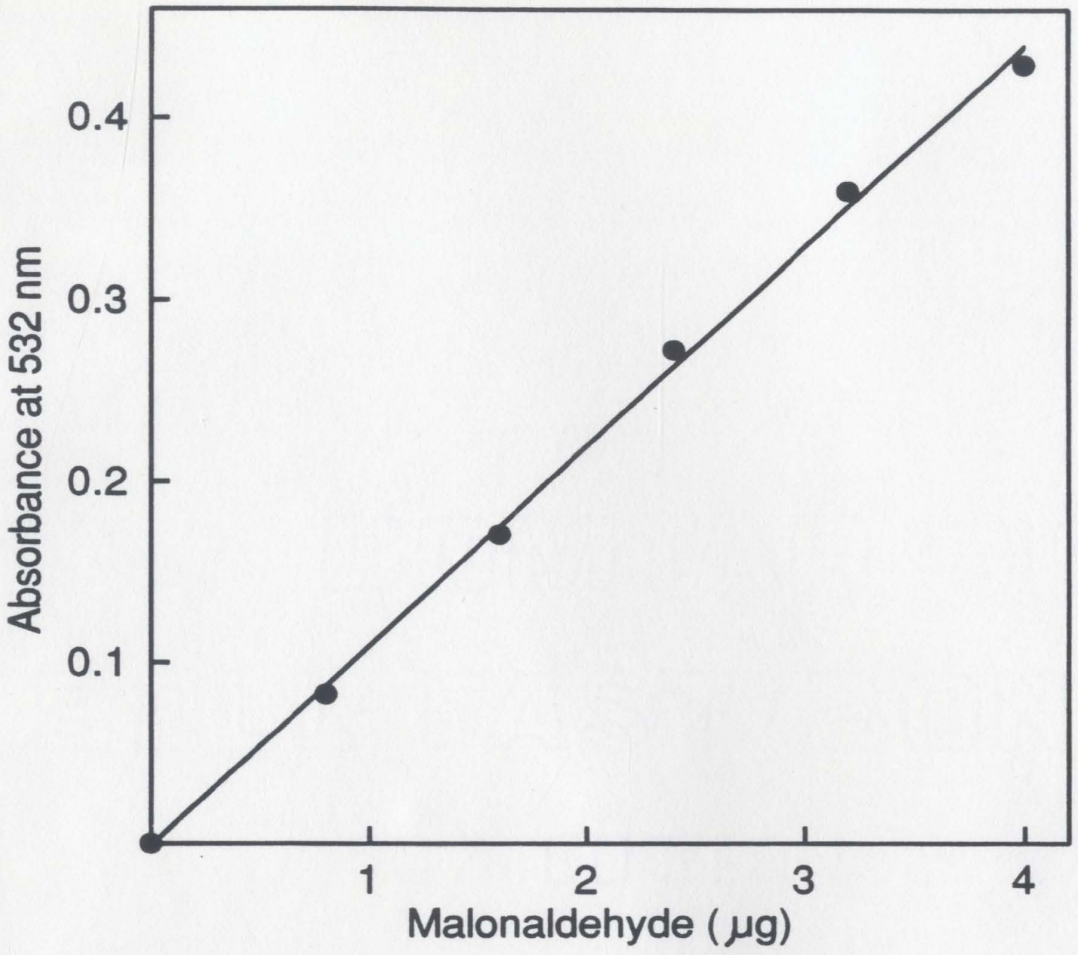

Figure A.6 Graph of standard line of 2-Thiobarbituric acid (TBA) concentration dependance on absorbance at 532 nm.

A.6 Thiobarbituric acid (TBA) value estimation

Regression coefficient (r) = 0.998
Equation of the line was Y = aX+b where,

Y = absorbance at 532 nm
X = concentration of malonaldehyde in 5 mL distillate µg (C)
a = 0.117
b = 0.0

$A_{532} = 0.117*C$
Therefore, $C = 8.54A_{532}$
Since W grams of sample was used for distilling 50 mL, the TBA value
$= 8.54A_{532} \times 10/W$ (mg malonaldehyde equivalents/kg sample).